PHYSIOLOGICAL STUDIES IN SEEDS: GERMINATION, SOIL ESTABLISHMENT, PRODUCTION

by

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THESIS presented for the Degree of

DOCTOR of PHILOSOPHY

University of Edinburgh

June 1933.

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Published Paper, from "Plant Physiology" (July 1933)

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DATE OF FLOWERING AS AFFECTED BY CLIMATIC TEMPERATURE

J. F. A. MACLAGAN

(WITH EIGHT FIGURES)

Introduction

The investigation reported here is mainly concerned with the effect of temperature on the flowering date of a number of genera of woody shrubs. Since the reproductive phase of a plant is not independent of its vegetative phase, and since temperature does not act independently of other climatic factors, before considering the special aspect of the problem,—the time of onset of the reproductive phase as influenced by temperature,—it seems advisable to examine briefly the wider question of how the plant as a whole is affected by climatic conditions.

INFLUENCE OF CLIMATIC FACTORS.—The effect on the plant of climatic conditions is not clear-cut. One climatic factor rarely if ever exercises an effect independently of other external factors, climatic, soil, etc. The reaction of the plant itself is complex. Climatic factors operate by their influence on the metabolism of the plant, and the reactions of the various metabolic functions are not uniform. These interacting units of the environment and the plant's metabolism affect flowering both as to quantity and time.

The primary effects of these climatic variations express themselves in a broader sense in the relationship which exists between the vegetative and reproductive phases of the plant. In this broader sense any factor which tends to stimulate the development and therefore the continuance of the vegetative phase must retard the onset of the reproductive phase. The date of first flowering may be regarded as the first marked outward indication of the onset of the reproductive phase. If this be accepted, then from its variation from year to year, any correlation which can be established between weather and such variation may be taken as evidence of the effect of climatic factors on the relationship between the two phases. Actually the formation of the first definite flower initial marks the initiation of the change from vegetative to reproductive phase. This being so, date of flowering is in reality a late indication of this change of phase. No data are available on this fundamental point regarding the time when change of phase commences. Following initiation of change of phase are many other stages in the march of events toward actual flowering. Most significant of these steps is the initiation and formation of the actual gametes. It will be indicated later that it is at the moment of gamete formation that temperature exerts its maximum effect.

Previous work has been concerned mainly with amount of flowering and fruit setting in each year, rather than with date of flowering. While the quantitative side of flowering is not necessarily affected in the same way as time, nevertheless it is related.

BARKER and LEES (2) dealt with the effect of weather on fruit bud formation at Long Ashton. What is called good "ripening" or "maturing" of the wood occurs when vegetative growth ceases somewhat early in autumn, thus favoring the formation of flower buds (change of phase) before winter sets in. The comparatively wet July and August at Long Ashton caused active vegetative growth to continue late into the autumn so long as the temperature was high enough to favor it, and fruit bud formation suffered in consequence.

LEES (4) in a later paper says: "When the influence of pests, diseases, manuring, pruning and stock influence are eliminated, the remaining factors of previous crop and summer rainfall determine the future crop in the large majority of cases."

INFLUENCE OF TEMPERATURE.—It must be clearly realized that temperature, although dealt with here almost as an isolated factor for the purposes of this investigation, cannot strictly be separated from the other components of the environment. Similarly, time of flowering is influenced not only directly by the weather but also indirectly by the other processes taking place in the plant. Hence a perfect correlation of temperature with time of flower opening is not to be expected, although, as will be shown later, temperature is of major importance.

Previous workers have used the summation of all temperatures above an arbitrary datum line over a certain period to express the total amount of effective heat incident, and so to determine the dependence of plant development on this factor (for example, LIVINGSTON 5, and PALLADIN 6). The results arrived at by such a method are unsatisfactory. Temperature above a certain point may be non-effective or deleterious; the usually depressant or positively injurious effect of frost is ignored; and no allowance is made for the physiological effects of change in temperature at different temperature levels. Simple change of temperature within short periods has been shown to have a profound effect on plant development, at least at germination.

FLOWERING DATE AS INFLUENCED BY TEMPERATURE.—In the present investigation the weather records and flowering records have been compared with a view to finding some period or periods of the year when weather has a marked and consistent correlation with date of flower opening. Temperature has been found to exhibit such a correlation. The mechanism through which the effect of temperature at particular times operates has been sought principally in the condition of the different parts of the flower bud, particularly the sporophylls, at these times. In this regard it is of interest to note that BALL (1), quoting GOFF as his authority, says in connection with the development of the flower buds of the different fruit trees: "The order of development is the same in the different fruits, *viz.*, first the calyx appears, then the petals, stamens, and finally the pistil. All these parts are present before the winter sets in, the ovules and pollen grains not being formed until February or March of the following year. There are exceptions to this since GOFF found that in the gooseberry ovules were generally present before the winter set in." BALL found that in the buds of three varieties of plum, Victoria, Monarch, and Pond's seedling, all the flower parts were present in November but ovules and pollen grains were not usually formed until January or February.

WHYTE (7) makes an illuminating observation in connection with the differentiation of floral parts. "The subject of time of reduction does not appear to have received much attention in the past, but from observations made by the writer on several unrelated genera of flowering plants, it may be said that in all those examined a considerable interval always occurs between the reduction processes in the pollen mother cells and the megaspore mother cells in any given flower. Growth of the female tissues does not generally commence until complete tetrads are formed, and pollen has been developed for some time before reduction in the ovules. The interval between the reduction process is probably governed largely by the amount of ovular development in the plant concerned."

COULTER and CHAMBERLAIN (3) mention the time of formation of the microsporangia and megasporangia for species in which the details are known. In the case of the microsporangia, different species exhibit considerable variation. They suggest that for those plants whose flowers open early in the season the mother-cell stage is the usual winter condition. At

any rate the interval of time between formation of the archesporium and pollination is probably in many cases considerably longer than is generally supposed.

In the winter bud of *Quercus velutina* the stamens are well formed but the tissue is still homogeneous. The microsporangia of *Salix glaucophylla*, *Populus monilifera*, and *Symplocarpus*, however, pass the winter in the mother-cell stage. A further advance is seen in *Alnus glutinosa* and *Corylus americana*, whose mid-winter catkins contain pollen ready for shedding, with the generative cell formed.

The length of time taken for the development of megasporangia is probably also very variable and related to the seasonal habit of the plant. CHAMBERLAIN has found that the megaspore mother cell of *Salix* and *Populus* is not formed until the winter dormancy of the plant is over and growth renewed in spring. In *Erythronium* the mother-cell stage is reached by the end of November and persists until early spring. The mother-cell stage has been found in *Acer rubrum* in March, indicating that in this species also the mother-cell is the condition in which the winter is passed. It is to be noted that in *Salix* and *Populus* the microsporangia pass the winter in the mother-cell stage, but the megaspore mother cell is not formed until spring, a considerable interval of time elapsing between the processes of formation in the two classes of spore, male and female.

Records of Data

The records of flowering dates on which this work is based are those kept at the Royal Botanic Garden, Edinburgh (unpublished). The records consist of lists of those plants which came into flower during each week of the year. Occasionally the entries have been made at fortnightly intervals instead of at the end of each week, and sometimes the period has been extended to three weeks, or even a month. Such extensions are most frequent in the case of early spring and late autumn records. The continuity of the records has been broken by the war and other causes, but sufficiently complete records are available for the years 1919 to 1929, excluding 1924. The temperature records used here are those taken at Edinburgh University and published in the monthly weather reports of the Meteorological Office, in conjunction with the daily weather records kept at the Royal Botanic Garden, Edinburgh (unpublished).

The data have been organized into graph form, and in each case the graph of the yearly flowering date has been plotted in juxtaposition to the graph of the significant weather belt or belts.[1] The coordinates of the temperature graph are: abscissae, years; and verticals, average temperature of significant belt. In the graphs of the date of flowering the abscissae are years, and the verticals are the deviation in each year from the average date of flowering over the period in question. The graphs are supplemented by tables giving in greater detail the data from which the graphs have been drawn.

[1] "Weather belt" is defined as the weather obtaining over a period of time, from one to three months, which by analysis has been found to be significant in causing, by its deviation from normal, a deviation from the normal flowering time of each species.

Consideration of weather data more detailed than monthly averages has not been attempted except in a very few instances. Not only the action of other external factors, but also the relative lack of accuracy of the flowering records would tend to invalidate any attempt at a finer correlation if a more detailed analysis were carried out.

As might be expected from a general knowledge of the annual periodicity of plants, correlations between weather and flowering date appear most clearly in autumn and spring. As pointed out, many species form the flower buds in the autumn of the year previous to their opening. In such species "wood maturation" will have an important effect on the subsequent year's flowering. Rainfall is of importance as well as temperature, since drought causes the cessation of vegetative growth and thus allows the reproductive phase to commence.

As different stages of development are attained in the flower buds of different species before winter sets in, and the final stages are accomplished when activity recommences in spring, the autumn weather probably affects different species to different extents. An almost complete theoretical graded series can be erected in connection with degree of development of the reproductive phase in different species in winter.

At one end of the series are species which overwinter with the buds in the purely vegetative condition, no attempt at differentiation being made. Perennials of this nature must be only a single stage more complicated in their weather response than annuals. While the weather prior to winter may affect their physiological state and therefore presumably their subsequent flowering, it will be the weather of the spring (and summer) immediately prior to flowering which will be significant.

The next stage in the series is that of perennials which proceed to differentiation of buds prior to the onset of winter, but which cease differentiation at some stage before reduction and spore formation. Such plants over-winter with two classes of buds, leaf buds and incipient flower buds. The weather prior to over-wintering must affect such differentiation and therefore flower production in the following year. How far this is significant cannot be stated. Gardeners believe that a plant with formed flower buds can be, by appropriate manipulation of external factors in early spring, induced to change its "intentions," in so far that the flower buds do not produce flowers but return to vegetative growth. Such reversal

might be possible in plants of the class falling into this grade, but it does not seem possible for plants of the next grade, which is that of perennials that over-winter with flower buds so differentiated that the sporophylls have assumed their definite character. A slightly more advanced stage is that when not only are the sporophylls definite in character, but the spores and gametes, at least on the microspore side, are definitely formed.

In perennials of the first class, autumn weather can have comparatively little effect on date of flowering in the following year. In those of succeeding classes, the weather of the autumn preceding any flowering year gains increasing importance as we ascend the series. In essence the statement may be made that, where the species proceeds in its development to a somewhat advanced stage of the reproductive phase before entering the winter dormant period, a significant weather belt must exist in the autumn prior to the flowering year. Furthermore, as has already been indicated from the literature, and as will be shown in actual cases later, since the change of phase usually occurs in two steps (male development prior to over-wintering and female development subsequent to over-wintering), two significant weather belts may exist. The weather belts coincide with the time of the respective steps in development. In the case of those plants which over-winter entirely in the vegetative phase, one major weather belt may be looked for in spring prior to flowering. Finally, the actual process of bud bursting and flower opening in all species must be conditioned to some extent by weather, and therefore another type of weather belt may be expected. The effect of frost on the opening buds is especially significant, more from the point of view of the records. The gardener observer, seeing frost-blasted buds, would probably not record the plant as having flowered, but would wait for a second crop of buds to open before entering the plant into the records. Three main significant weather belts may therefore be envisaged: (1) a belt occurring in autumn after one year's flowers have fallen and far removed in time from the next flower opening, called here the *distal belt*; (2) another belt occurring in spring or early summer, nearer in time to date of flowering, called here the *proximal belt*; and (3) the weather just at or about flowering time, called here the *immediate belt*.

Soon after the work was commenced it was realized that, owing to the method of compiling the flowering records at intervals of a week or more, the experimental error in dealing with single species might be so large as to obscure the clear outlines. Consequently the genus was taken as the unit for comparison in order to establish the general rules governing its behavior if such existed. Only those species in each genus which were completely recorded were considered and incorporated. While it was felt that the members of a genus were related sufficiently closely to be thus dealt with as a unit, the possibility of single species showing individuality has not been lost sight of, and these are detailed when they occur.

While in general the results obtained point to temperature as a master factor in flower-bud development, particular cases often cannot be explained on this basis. This is to be expected, however, in view of the fact that other factors come into play and may have a modifying, and sometimes an overriding, effect. For example, rainfall would affect the nitrate content of the soil and thus (or even by modified water supply) affect the interrelationship between the vegetative and reproductive phases. Internal factors may also have a potent, although less easily defined, influence. For example, it is generally accepted that a year of profuse flowering is followed in the subsequent year by a paucity of flowers, an effect which seems to hold despite favorable external conditions in the second year. The question of characters of a genetical nature cannot be considered here, and in any case the clear lines of their possible effect are blurred by the nature of the records. Further, the question of imposed rhythm might be pertinent, but the fact that the records of most species used are of long standing militates against this.

Presentation of data

RHODODENDRON

It was realized at the commencement of the analyses that "maturation of the wood" in autumn is an important feature of this genus. A comparison of the autumn conditions, however, failed to show any correlation with time of flowering. On further study of the records it became clear that a weather belt occurs rather earlier in the year, about one to two months after flowering, which can be correlated with date of flowering in the following year. Unpublished data supplied by Miss F. B. MURRAY to the effect that the formation of pollen in the next year's flower buds may reach an advanced stage in development at a comparatively early date, within some two months after flower fall, strengthens this assumption, especially in view of the graded series already indicated. A striking case is seen in *Rhododendron souliei*, which in 1930 came into flower in the first week of June, and had flower buds with pollen formed by the first week of July.

As the species of *Rhododendron* considered here flower over a period of five months (February to June), the distal weather belt is not the same for all; hence the genus has been divided into three groups according to the time of flowering of the species. Each group consists of species which on the average flower practically together.

While the temperature at this distal weather belt showed a decided influence on time of flowering, it was found that in all species the correlation failed to hold in the years 1922 and 1923. This was accounted for when a proximal weather belt was taken into consideration. The average temperature of the subsequent January–March period was found to provide an explanation of these aberrations when it emerged as the proximal weather belt. Cytological evidence again supported the contention, for though the pollen may reach an advanced stage in the autumn, the development of the ovary does not take place until the spring. Indeed, reduction division may not take place until a very short time before flowering (unpublished data by Miss MURRAY).

Graphs are presented to illustrate this combined effect on time of flowering of the temperature at two different periods. In each group of species the most fully recorded species has been chosen to represent the group. In the graph of temperature for the distal belt, 1918 temperature has been plotted on the same vertical as flowering for 1919, and so on, in order to facilitate direct comparison.

In addition to the graphs, tables are appended giving the complete data for all members of each group.

TABLE OF RHODODENDRON SPECIES

SPECIES	AVERAGE FLOWERING DATE
GROUP I:	
R. barbatum	February 28
GROUP II:	
R. metternichii	March 23
R. ciliatum	March 28
GROUP III:	
R. schlippenbachii	April 29
R. vaseyi	May 4
R. roylei	May 10
R. decorum	May 11
R. smirnowii	May 20

Group I: Species flowering in February

Although only one species falls into this group, its agreement with the general behavior of the genus seems to justify its inclusion.

The distal temperature belt is April/May, the proximal temperature belt January/February. Figure 1 and table I refer to this group.

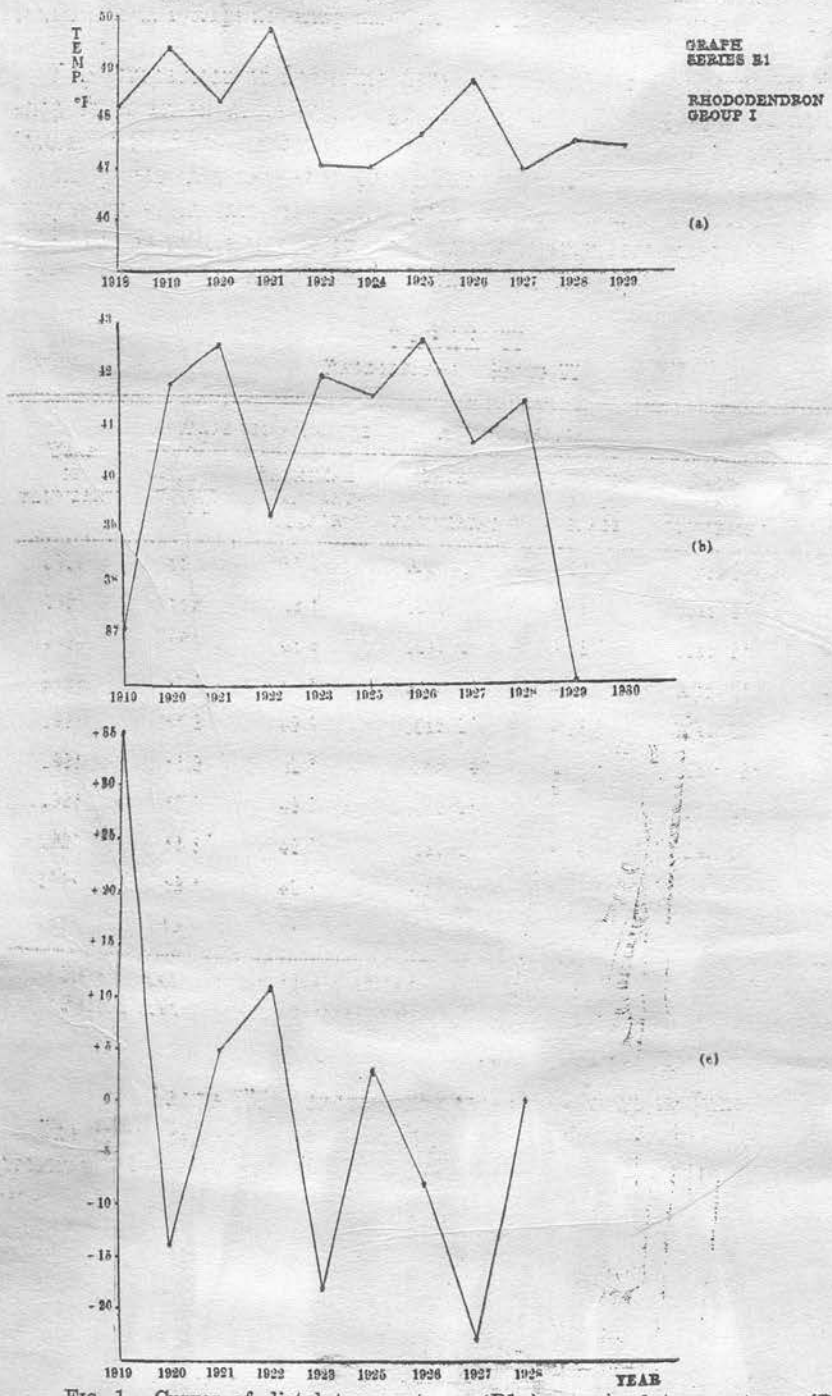

Fig. 1. Curves of distal temperatures (R1a), proximal temperatures (R1b), and deviations from average flowering time (R1c), of *Rhododendron barbatum*, 1918–1929.

Comparing first the graphs of flowering time (fig. R1c) and April–May temperature of the preceding year (fig. R1a), it will be seen that, in general, high temperature corresponds to early and low temperature to late flowering, although this belt is separated from the actual flowering date by nearly nine months. In this species, however, as in most other species of the genus, in the years 1922 and 1923 the reverse was true, that is, flowering in 1922 was late and in 1923 early, while the temperature of the distal belt in 1921 was high and in 1922 low. Again in the year 1919 flowering was very late although temperature of the distal belt was average. Comparing the temperature graphs of the distal and proximal belts (fig. R1a and R1b), it will be seen that they agree except in those years of aberration 1919, 1922, and 1923. In other words, the proximal temperature had an overriding effect in these years. The extremely cold spring (proximal belt) of 1919 caused flowering to be late in that year, in spite of average warmth in the distal belt (April–May, 1918). The cold spring of 1922 had the same effect, and the comparatively warm spring (proximal belt) of 1923 made flowering early in that year in spite of the less-than-average temperature of the previous April–May (distal belt).

TABLE I

Rhododendron, Group I

Year of flowering	Average temperature		*R. barbatum*	
	Distal (April–May)	Proximal (January–February)	Date of flowering	Deviation from average
1919	48.20	37.05	Apr. 5	+35.0
1920	49.35	41.75	Feb. 14	−14.0
1921	48.30	42.50	Mar. 5	+ 5.0
1922	49.70	39.20	Mar. 11	+11.0
1923	47.05	41.90	Feb. 10	−18.0
1925	47.00	41.50	Mar. 7*	+ 3.5
1926	47.65	42.60	Feb. 20	− 8.0
1927	48.70	40.60	Feb. 5	−23.0
1928	46.95	41.40	Mar. 3	+ 0.5
1929	47.50	36.00	.....	.....

* Record made at end of a fortnight.

Group II: Species flowering in March

For this group the distal temperature belt is June, the proximal temperature belt January/March. Figure 2 and table II refer to this group.

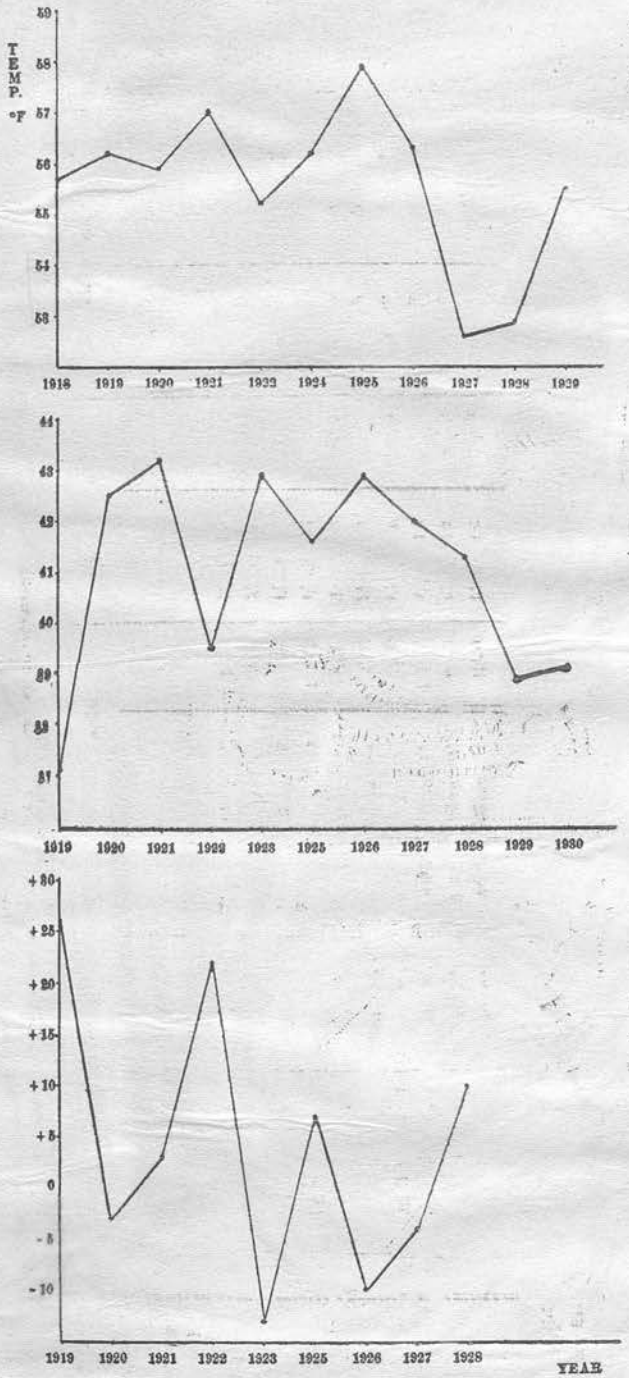

Fig. 2. Curves of distal temperatures (R2a), proximal temperatures (R2b), and deviation from average flowering time (R2c), of *Rhododendron mettermichii*, 1918–1929.

For comparison of flowering date and weather, *R. metternichii* has been adopted as an example and its graph drawn. If the graphs of figure R2a (distal belt), R2b (proximal belt), and R2c (flowering curve) are compared, it will appear that here as in group I the correlation of flowering with distal temperature (in this case June) holds except in the years 1919, 1922, and 1923; and as in the case of group I, this is clearly accounted for by the temperature which obtained in the proximal belt affecting these years.

TABLE II

Rhododendron, Group II

Year of flowering	Average temperature		*R. metternichii*		*R. ciliatum*	
	Distal (June)	Proximal (January–March	Date of flowering	Deviation from average	Date of flowering	Deviation from average
1919	55.7	37.1	Apr. 19	+26.0	Apr. 19	+21.0
1920	56.2	42.5	Mar. 6	− 3.0	Mar. 20	− 8.0
1921	55.9	43.2	Mar. 19	+ 3.0	Mar. 26	− 2.0
1922	57.0	39.5	Apr. 8	+22.0	Apr. 15	+17.0
1923	55.2	42.9	Mar. 10	−13.0	Mar. 10	−18.0
1925	56.2	41.6	Apr. 4*	+ 7.5	May 2†	.....
1926	57.9	42.9	Mar. 13	−10.0	Mar. 13	−15.0
1927	56.3	42.0	Mar. 19	− 4.0	Mar. 19	− 9.0
1928	52.6	41.3	Apr. 7*	+10.5	Apr. 7*	+ 5.5
1929	52.9	38.9	.....	.....	.....	.....

* Record made at end of a fortnight.

† Record made at end of three weeks.

Group III: Species flowering in April and May

The distal temperature belt affecting this group is June/July, and the proximal temperature belt is January/March. Figure 3 and table III refer to this group.

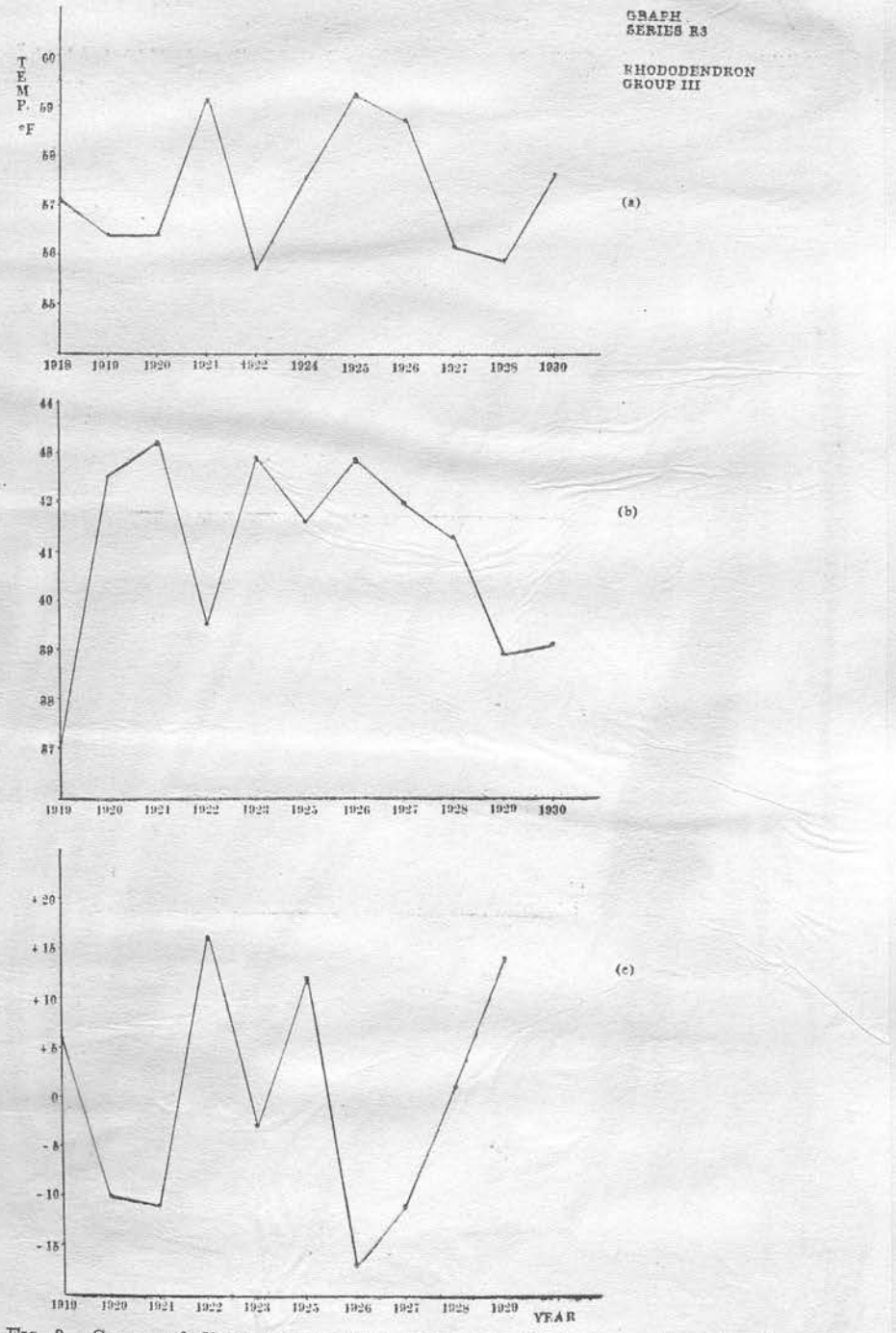

Fig. 3. Curves of distal temperatures (R3a), proximal temperatures (R3b), and deviation from average flowering time (R3c), of *Rhododendron vaseyi*, 1919–1929.

R. vaseyi has been chosen to exemplify this group, and graphed for purposes of comparison with the temperature belts. Figures R3a (distal temperature), R3b (proximal temperature), and R3c (flowering date) show the same features as the graphs for groups I and II.

TABLE III

Rhododendron, Group III

A, date of flowering; B, deviation from average date of flowering

Year of flowering	Average temperature		*R. schlippenbachii*		*R. vaseyi*		*R. roylei*		*R. decorum*		*R. smirnowii*	
	Distal (June–July	Proximal (January–March)	A	B	A	B	A	B	A	B	A	B
1919	57.1	37.1	May 17	+18.0	May 10	+6.0	May 17	+7.0	May 17	+6.0	May 24	+4.0
1920	56.4	42.5	Apr. 24	−5.0	Apr. 24	−10.0	Apr. 17	−23.0	May 1	−10.0	May 8	−12.0
1921	56.4	43.2	.....	.....	Apr. 23	−11.0	May 7	−3.0	May 7	−3.0	May 7	−13.0
1922	59.1	39.5	May 13	+14.0	May 20	+16.0	May 20	+10.0	May 20	+9.0	May 27	+7.0
1923	55.7	42.9	May 5*	+2.5	May 5*	−2.5	May 5*	−8.5	June 2	+22.0	May 19	−1.0
1925	57.55	41.6	.....	.....	May 16	+12.0	May 25	+15.0	May 2†	.....	May 25	+5.0
1926	59.2	42.9	Apr. 10	−19.0	Apr. 17	−17.0	Apr. 17	−23.0	May 1	−10.0	May 29	+9.0
1927	58.7	42.0	Apr. 16	−13.0	Apr. 23	−11.0	May 21	+11.0	Apr. 16	−25.0	May 7	−13.0
1928	56.15	41.3	.....	.....	May 5	+1.0	May 12	+2.0	May 19	+8.0	May 19	−1.0
1929	55.9	38.9	.....	.....	May 18	+14.0	May 18	+8.0	May 18	+7.0	June 1*	+8.5

* Record made at end of a fortnight.

† Record made at end of three weeks.

CYTISUS

This genus provides a contrast to *Rhododendron*, for in the case of *Cytisus* no distal temperature belt affecting time of flowering could be discovered. An examination of the flower buds of a number of species was made toward the end of February and no pollen was found. If, as seems likely, the flower bud passes the winter with the stamens and ovary in an undifferentiated condition, it is not to be expected that the autumn weather will have any marked effect on time of flowering. Thus in *Cytisus* no distal weather belt will occur.

TABLE OF CYTISUS SPECIES

SPECIES	AVERAGE FLOWERING DATE
C. kewensis	April 20
C. beanii	April 21
C. ardoinii	April 21
C. biflorus	April 29
C. purgans	May 7
C. decumbens	May 8
C. hirsutus	May 12
C. albus	May 13
C. horniflorus	May 15
C. versicolor	May 17
C. glabrescens	May 21
C. purpureus incarnatus	May 22
C. scoparius	May 23
C. scoparius prostratus	May 23
C. scoparius Andreanus	May 26
C. purpureus albus	May 28
C. sessifolius	May 29

CYTISUS, ALL SPECIES

The data show the month of March as the period during which temperature exercises its particular influence. Figure 4 illustrates this point.

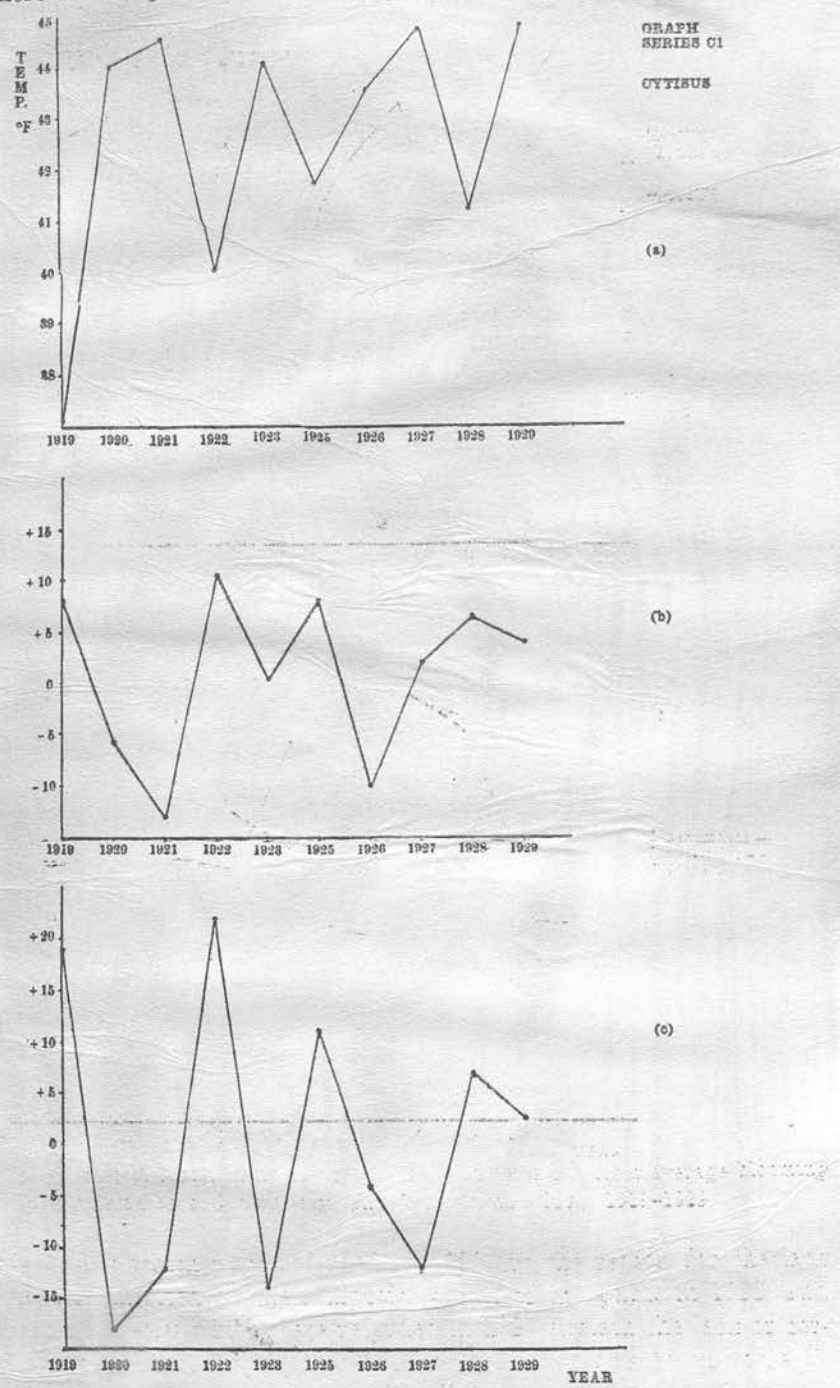

FIG. 4. Curves of March temperatures (C1a), composite deviation from average flowering time for 7 species of cytisus (C1b), and deviation from average flowering time for *Cytisus beanii* (C1c), 1919–1929.

Figure 4, C1a, shows March temperature, and C1b is a composite graph for seventeen species of *Cytisus*. This composite graph is compounded from the average of the figures representing the deviations from the average flowering date shown in each year by each of the seventeen species. The figures for each individual species are given in table IV, columns 3 to 36, and the figures derived from these, from which the composite graph has been drawn, in the last column of the same table. In finding the average for 1920, the figure for *C. horniflorus* has been ignored, since if it were included its enormous deviation (−70) would give the average a false value. The two curves *a* and *b* show a remarkably close correlation, except that in the years 1923, 1927, and 1929 flowering was rather late. These deviations will be explained later by a more detailed consideration. Following on these is a graph (fig 4, C1c) for a single species, *Cytisus beanii*, which

shows a remarkably perfect correlation with the temperature curve. As will be seen in table IV, the other species behave in the same way with minor deviations.

Cytisus as a whole seems to be rather an unstable genus, or perhaps easily affected by external conditions not evaluated here. There are a number of aberrations occurring in single species for which an explanation cannot be attempted. Such aberrations are very marked in the year 1929, and are the cause of lack of correlation between the composite graph (fig. 4, C1b) and the March temperature graph (fig. 4, C1a) in that year.

While the data do not permit explanation of these minor departures from the rule, two major departures may be accounted for on the general thesis developed here. These two deviations are seen, one in 1923 and the other in 1927. In 1923 three species, *C. albus, C. horniflorus,* and *C. glabrescens,* differed from the others in flowering very late.

Species	Average flowering date	Flowering date in 1923
C. albus	May 13	June 2
C. horniflorus	May 15	June 23
C. glabrescens	May 21	June 16

This is illustrated in figure 5.

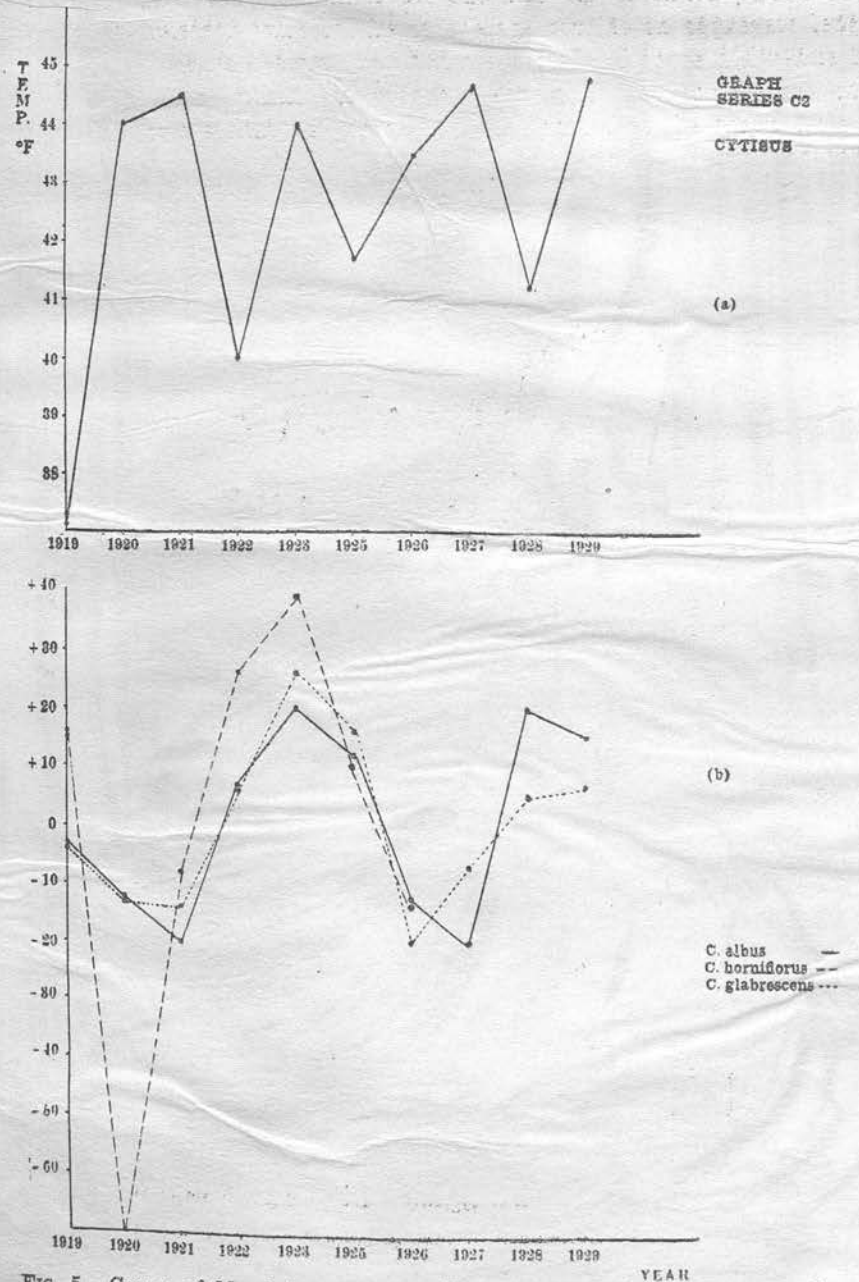

FIG. 5. Curves of March temperatures (C2a), and deviation from average flowering time for *Cytisus albus, C. horniflorus,* and *C. glabrescens* (C2b), 1919–1929.

In 1927 other three species, *C. decumbens, C. versicolor,* and *C. scoparius,* flowered later than their fellows.

Species	Average flowering date	Flowering date in 1927
C. decumbens	May 8	May 28
C. versicolor	May 17	May 28
C. scoparius	May 23	June 4

This is illustrated in figure 6.

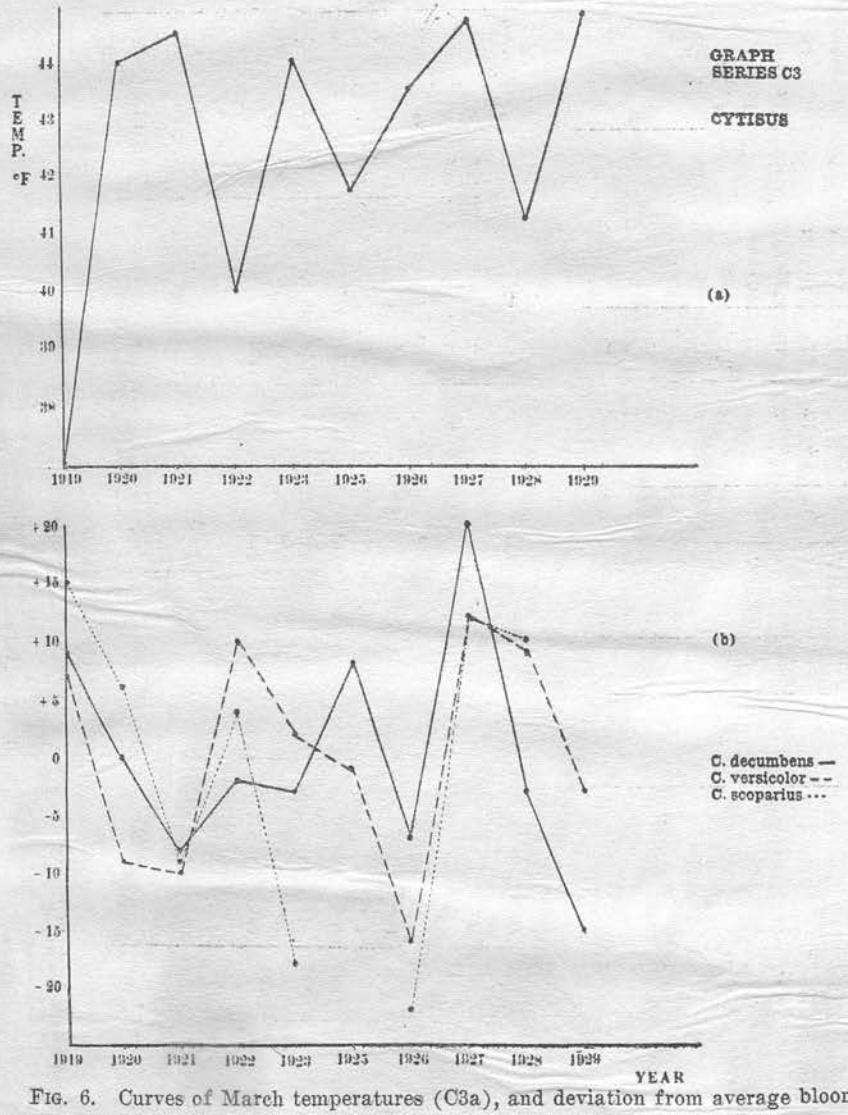

FIG. 6. Curves of March temperatures (C3a), and deviation from average blooming time for *Cytisus decumbens, C. versicolor,* and *C. scoporius* (C3b), 1919–1929.

Those species which were retarded in 1923 are the reason for the lack of correlation exhibited by the composite graph of the genus in 1923, and similarly those species which were retarded in 1927 explain the lack of correlation of the composite graph in 1927. This is illustrated in table V, where alongside the composite figures for all seventeen species (column A) are seen two sets of composite figures for fourteen species (columns B and C), where in one case the three species which were late in 1923, and in the other case the three species which were late in 1927, have been omitted. It will be seen that these columns (B and C) show their greatest deviation from column A in 1923 and 1927, respectively. If the figure in column A is replaced in 1923 by the figure in column B, and in 1927 by the figure in column C, the disturbing influence of these retardations, caused, as will be seen later, by cold spells, will be eliminated, and the A figures will be seen to fluctuate in accordance with the March temperature in a much more consistent manner.

The retardations of these species are examples of the effect of cold weather just at time of flowering (immediate temperature belt). In 1923 a cold spell set in on May 9 and lasted until the end of the month. The average temperature for May in that year was 47.5° F., several degrees below the usual May temperature. In 1927, in the last week of April, there were severe ground frosts, sufficient to retard flowering. The grass minimum temperatures recorded at the Royal Botanic Garden, Edinburgh, for this period, are:

	°F.
April 26	23
April 27	18
April 28	14.5
April 29	21
April 30	24
May 1	29

Considering these data as a whole, it is clear that in *Cytisus* there are two belts of weather (proximal and immediate) during which temperature affects date of flowering. The mechanism through which the temperature operates is not precisely known, and must await a cytological investigation. It may be that the two periods synchronize with spore differentiation; the earlier period with microspore development, and the later with megaspore production. This implies the existence of a time interval between the two processes which remains to be proved. On the face of the data, it seems likely that there is no definite cessation of activity at an intermediate point, but rather that the two processes are continuous, the one following the other without a definite interval; but that this extended period offers an extended time for temperature to operate, similar in aggregate length to that in the species showing two definite periods of spore differentiation activity, and that within this aggregate period the temperature may influence the two significant processes. The fact that the sharp lines of spore differentiation activity are in this way somewhat blurred accounts for the apparently fickle nature of the genus as a whole.

TABLE IV

CYTISUS

A, DATE OF FLOWERING; B, DEVIATION FROM AVERAGE DATE OF FLOWERING

YEAR OF FLOWERING	AVERAGE TEMPERATURE (MARCH)	C. KEWENSIS ...		C. BEANII		C. ARDOINII		C. BIFLORUS ...		C. PURGANS		C. DECUMBENS		C. HIRSUTUS		C. ALBUS	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1919	37.1	Apr. 26	+6	My. 10	+19	My. 3	+12	My. 10	+11	My. 17	+10	My. 17	+9	.....	.....	My. 10	–3
1920	44.0	Apr. 10	–10	Apr. 3	–18	Apr. 17	–4	Apr. 24	–5	Apr. 24	–13	My. 8	0	My. 22	+10	My. 1	–12
1921	44.5	Mar. 26	–25	Apr. 9	–12	Apr. 2	–19	Apr. 9	–20	Apr. 30	–7	Apr. 30	–8	Apr. 23	–19	Apr. 23	–20
1922	40.0	Apr. 29	+9	My. 13	+22	My. 6	+15	My. 13	+14	My. 20	+13	My. 6	–2	Ju. 3	+22	May 20	+7
1923	44.0	Apr. 7	–13	Apr. 7	–14	Apr. 7	–14	Apr. 14	–15	Apr. 21	–16	My. 5	–3	My. 5	–7	Jn. 2	+20
1925	41.7	My. 2	+12	My. 2	+11	My. 2	+11	My. 9	+10	My. 2	–5	My. 16	+8	Jn. 6	+25	My. 25	+12
1926	43.5	My. 1	+11	Apr. 17	–4	Apr. 17	–4	My. 1	+2	My. 1	–6	May 1	–7	Apr. 24	–18	My. 1	–12
1927	44.7	Apr. 23	+3	Apr. 9	–12	Apr. 23	+2	Apr. 30	+1	My. 14	+7	My. 28	+20	My. 14	+2	Apr. 23	–20
1928	41.2	Apr. 21	+1	Apr. 28	+7	Apr. 28	+7	My. 5	+6	.....	.....	My. 5	–3	My. 5	–7	Jn. 2	+20
1929	44.8	Apr. 27*	+3.5	Apr. 27*	+2.5	Apr. 27*	+2.5	Jn. 1*	–1.5	Jn. 1*	+21.5	Apr. 27*	–14.5	Jn. 1*	+16.5	Jn. 1*	+15

C. HORNIFLORUS		C. VERSICOLOR		C. PURPUREUS INCARNATUS		C. GLABRESCENS		C. SCOPARIUS		C. SCOPARIUS PROSTRATUS		C. SCOPARIUS ANDREANUS		C. PURPUREUS ALBUS		C. SESSIFOLIUS		AVERAGE OF B COLUMNS
A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
My. 31	+16	My. 24	+9	My. 31	+9	My. 17	–4	Jn. 7	+15	My. 24	+1	Jn. 14	+19	My. 31	+3	My. 31	+2	+8.25
Mar. 6	(–70)	My. 8	–9	My. 22	0	My. 8	–13	My. 29	+6	My. 8	–15	My. 22	–4	My. 22	–6	My. 29	0	–5.75
My. 7	–8	My. 7	–10	My. 14	–8	My. 7	–14	My. 14	–9	My. 28	+5	My. 7	–19	My. 14	–14	My. 14	–15	–13.00
Jn. 10	+26	My. 27	+10	Jn. 3	+12	My. 27	+6	My. 27	+4	Jn. 3	+11	My. 27	+1	Jn. 3	+6	Jn. 3	+5	+10.5
Jn. 23	+39	My. 19	+2	Jn. 2	–3	Jn. 16	+26	My. 5	–18	Jn. 2	+10	.....	.....	Jn. 2	+5	Jn. 2	+4	+0.25
My. 25	+10	My. 16	–1	Jn. 6	+3	Jn. 6	+16	.....	.....	My. 25	+2	My. 25	–1	Jn. 6	+9	Jn. 6	+8	+8.00
My. 1	–14	My. 1	–16	My. 22	–21	My. 1	–20	My. 1	–22	My. 1	–22	My. 29	+3	My. 22	–6	My. 15	–14	–10.00
.....	.....	My. 28	+11	My. 28	+6	My. 14	–7	Jn. 4	+12	My. 28	+5	My. 28	+2	My. 28	0	My. 28	–1	+2.00
.....	.....	My. 26	+9	Jn. 2	+4	My. 26	+5	Jn. 2	+10	My. 26	+1	Jn. 16*	+17.5	Jn. 2	+5	Jn. 16*	+14.5	+6.50
.....	.....	My. 18*	–2.5	Jn. 1*	–7.5	Jn. 1*	+7.5	.....	.....	.....	.....	Jn. 1*	+2.5	Jn. 1*	+0.5	Jn. 1*	–0.5	+4.00

* Record made at end of a fornight.

TABLE V

Cytisus

A, AVERAGE OF DEVIATIONS OF ALL 17 SPECIES FOR YEAR IN QUESTION; B, AVERAGE OF DEVIATIONS OF ALL SPECIES EXCEPT THOSE LATE IN 1923 (C. ALBUS, C. HORNIFLORUS, C. GLABESCENS) FOR YEAR IN QUESTION; C, AVERAGE OF DEVIATIONS OF ALL SPECIES EXCEPT THOSE LATE IN 1927 (C. DECUMBENS, C. VERSICOLOR, C. SCOPARIUS) FOR YEAR IN QUESTION.

YEAR OF FLOWERING	AVERAGE TEMPERATURE (MARCH)	A	B	C
1919	37.1	+ 8.25	+ 9.50(1.25)*	+ 7.75(0.5)
1920	44.0	− 5.75	− 5.00(0.75)	− 7.00(1.25)
1921	44.5	−13.00	−12.75(0.25)	−14.00(1.0)
1922	40.0	+10.50	+10.00(0.5)	+12.00(1.5)
1923	44.0	0.00	− 6.25(6.25)	+ 1.50(1.5)
1925	41.7	+ 8.00	+ 7.00(1.0)	+ 8.75(0.75)
1926	43.5	−10.00	− 9.00(1.0)	− 9.00(1.0)
1927	44.7	+ 2.00	+ 4.00(2.0)	− 1.00(3.0)
1928	41.2	+ 6.50	+ 5.50(1.0)	+ 7.50(1.0)
1929	44.8	+ 4.00	+ 3.00(1.0)	+ 5.25(1.25)

* Figures in brackets indicate difference between this value and the value for A.

SYRINGA

When the table of deviations from average flowering date for this genus was drawn up, it showed a high degree of consistency for the genus as a whole. As in *Cytisus,* no distal temperature belt was discernible, neither at first glance could a satisfactory proximal belt be found. The explanation was discovered in the marked overriding effect of the temperature at time of flowering (immediate temperature belt). When this belt was taken into consideration, *Syringa* was found to be governed, like *Cytisus,* by a proximal temperature belt in March, overriden by immediate temperature belts in April and May. Hence, although not at first evident, *Syringa* and *Cytisus* are very similar, although the immediate temperature belt has a more marked effect on *Syringa* than on *Cytisus.*

The twelve species of *Syringa* here considered fall into two classes according to their average time of flowering, hence in considering the genus two immediate temperature belts must be taken into account. The two species which have average flowering dates about the end of April/beginning of May (*S. villosa* var. *giraldii* and *S. pinnatifolia*) are governed by an immediate temperature belt in April. The other ten species, with average flowering dates at the end of May/beginning of June, are governed by an immediate temperature belt in May.

TABLE OF SYRINGA SPECIES

SPECIES	AVERAGE FLOWERING DATE
GROUP I:	
S. villosa giraldii	April 22
S. pinnatifolia	May 4
GROUP II:	
S. vulgaris	May 24
S. vulgaris alba	May 25
S. persica	May 25
S. villosa lutece	June 1
S. villosa	June 1
S. reflexa	June 6
S. yunnanensis	June 10
S. adamiana	June 13
S. emodii	June 17
S. emodii variegata	June 25

GROUP I: SPECIES FLOWERING IN LATE APRIL AND EARLY MAY

For these species March is the proximal temperature belt, and April the immediate temperature belt. Figure 7 and table VI refer to this group.

Examining figure 7, it will be seen that the curves of *S. villosa* var. *giraldii* and *S. pinnatifolia* (S1c), excluding *S. pinnatifolia* in 1923, follow the March temperature curve S1a rather consistently, the most significant deviations occurring in 1927 and 1929. Time of flowering in 1927, and

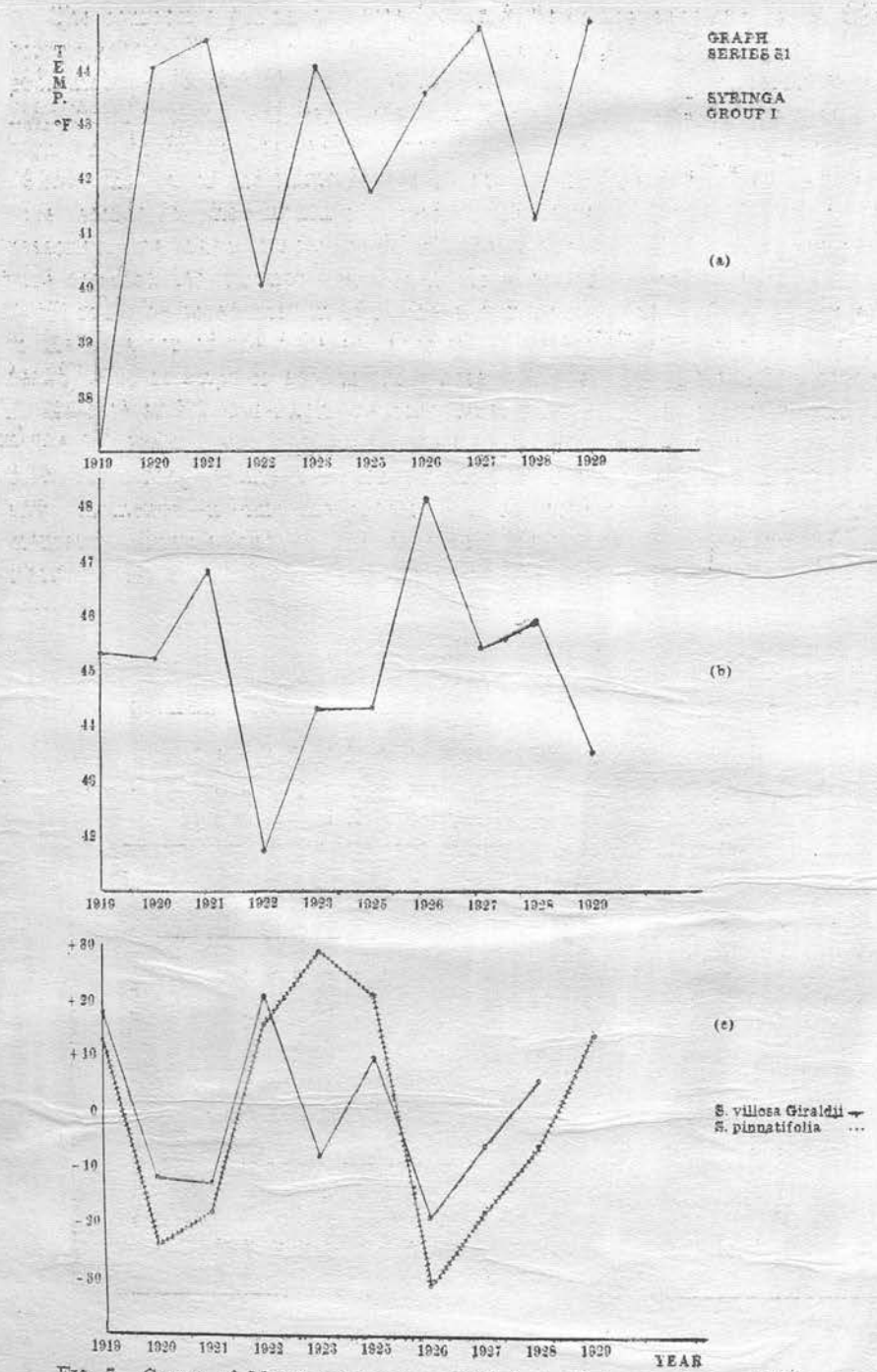

FIG. 7. Curves of March temperatures (S1a), April temperatures (S1b), and the deviation from average flowering time of *Syringa villosa giraldii*, and *S. pinnatifolia* (S1c), 1919–1929.

more especially in 1929, was later than might be expected from the high temperature during March in those years. This is accounted for by the overriding effect of the April temperature (immediate belt), which in 1927 was average and in 1929 low. The only other years in which the April temperature curve (S1b) deviates from the March temperature curve (S1a) are 1919 and 1923. Comparison with the curve of flowering time (S1c) shows that in the former year April temperature has slightly modified the effect of March temperature. A point of more interest is the effect of the temperature in April, 1923, which was slightly below average. Apparently it had no effect on *S. villosa* var. *giraldii*, and a disproportionately powerful effect on *S. pinnatifolia*. A more detailed study of the weather conditions during April and May supplies the explanation. The temperature during the early part of April, although somewhat low, was not sufficiently low to retard the earlier flowering species *S. villosa* var. *giraldii*, which in consequence of a warm March flowered by April 14. Subsequent sharp frosts on April 15, 18, 27, and 28 checked *C. pinnatifolia* and held up its flowering so that it came under the influence of an extremely cold period which set in on May 3. Thus *S. pinnatifolia* in 1923 was four weeks late in flowering, and in this year should really rank as one of the later-flowering group with an immediate temperature belt in May.

TABLE VI

Syringa, Group I

Year of flowering	Average temperature		*S. villosa giraldii*		*S. pinnatifolia*	
	March	April	Date of flowering	Deviation from average	Date of flowering	Deviation from average
1919	37.1	45.3	May 10	+18	May 17	+13
1920	44.0	45.2	Apr. 10	−12	Apr. 10	−24
1921	44.5	46.8	Apr. 9	−13	Apr. 16	−18
1922	40.0	41.7	May 13	+21	May 20	+16
1923	44.0	44.3	Apr. 14	− 8	June 2	+29
1925	41.7	44.3	May 2	+10	May 25	+21
1926	43.5	48.1	Apr. 3	−19	Apr. 3	−31
1927	44.7	45.4	Apr. 16	− 6	Apr. 16	−18
1928	41.2	45.9	Apr. 28	+ 6	Apr. 28	− 6
1929	44.8	43.5	.....	.....	May 18	+14

GROUP II: SPECIES FLOWERING IN LATE MAY AND JUNE

Here the proximal temperature belt is March and the immediate temperature belt May. Figure 8 and table VII refer to this group.

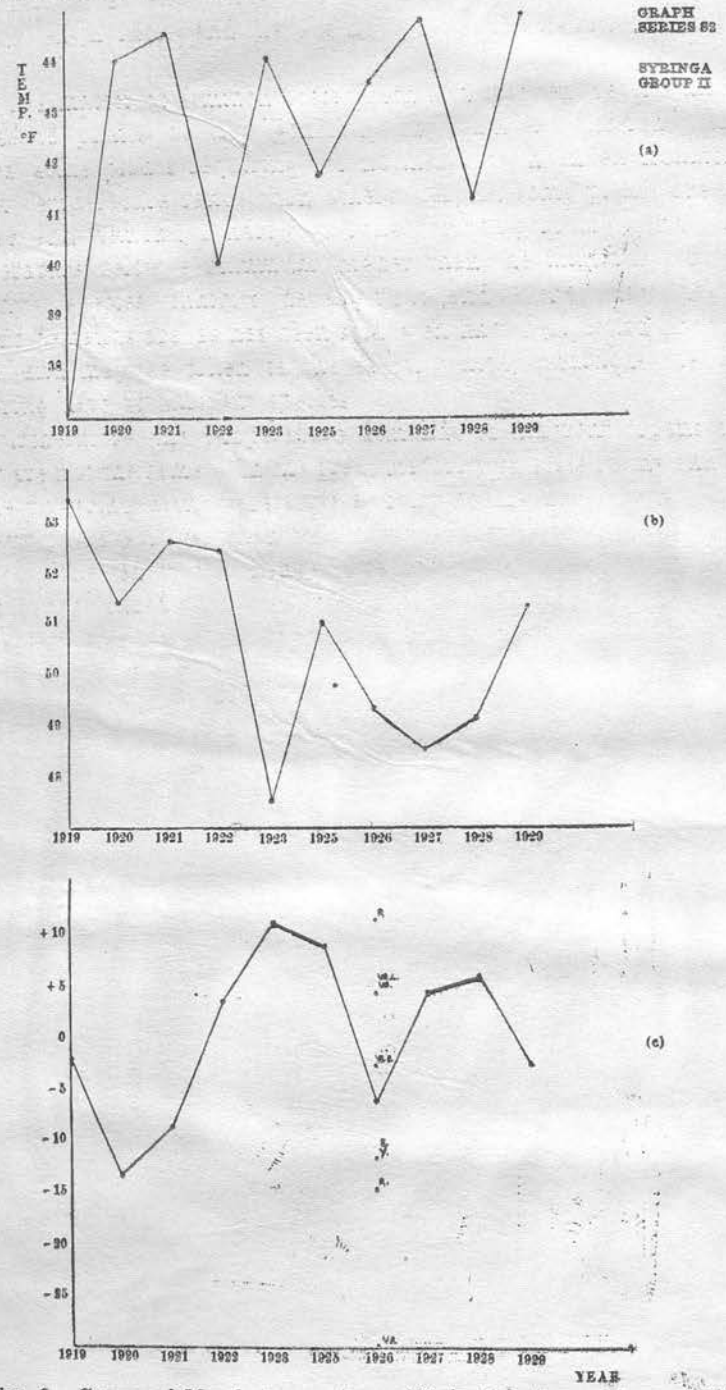

FIG. 8. Curves of March temperatures (S2a), May temperatures (S2b), and composite deviation from average blooming time for 10 species of *Syringa* (S2c), 1919–1929.

The composite graph of these ten later-flowering species (fig. 8, S2c) corresponds with the curve of March temperature (S2a); that is, of the proximal belt, except in 1923 and 1927. In 1923, March temperature was high but flowering was very late. In 1927, March temperature was also high, and flowering was again rather late. The graph of May temperature (S2b); that is, of the immediate belt, shows that the two lowest temperature records for that month occurred in the years 1923 and 1927, and may reasonably be taken to account for the deviations. In 1919, May temperature has modified but not overridden the effect of March temperature.

TABLE VII

Syringa Group II

A, date of flowering; B, deviation from average date of flowering

Year of Flowering	Average temperature		*S. vulgaris*		*S. vulgaris alba*		*S. persica*		*S. villosa lutea*		*S. villosa*	
	March	May	A	B	A	B	A	B	A	B	A	B
1919	37.1	53.4	May 24	0	May 24	–1	May 24	–1	.....	.....	May 31	–1
1920	44.0	51.4	May 22	–2	May 8	–17	May 15	–10	May 15	–17	May 15	–17
1921	44.5	52.6	May 14	–10	May 14	–11	May 14	–11	June 4	+3	May 14	–18
1922	40.0	52.4	June 3	+10	May 27	+2	May 27	+2	June 3	+2	June 3	+2
1923	44.0	47.5	June 9	+16	June 2	+8	June 2	+8	June 9	+8	June 9	+8
1925	41.7	51.0	June 6	+13	June 6	+12	May 26	0	June 6	+5	June 20*	+15.5
1926	43.5	49.3	April 24	–30	May 22	–3	June 5	+11	June 5	+4	June 5	+4
1927	44.7	48.5	June 4	+11	June 4	+10	May 21	–4	.....	.....	June 4	+3
1928	41.2	49.1	May 19	–5	May 26	+1	May 26	+1	.....	.....	.....	.....
1929	44.8	51.3	May 18	–6	June 1*	+3.5	June 1*	+3.5	June 1*	+3.5	.....	.....

	S. reflexa		*S. yunnanensis*		*S. adamiana*		*S. emodii*		*S. emodii variegata*		Average of B columns
	A	B	A	B	A	B	A	B	A	B	
1919	.....	.....	June 14	+4	June 14	+2	June 7	–10	June 14	–11	–2.25
1920	May 29	–8	May 22	–19	May 22	–21	May 29	–19	June 19	–6	–13.5
1921	.....	.....	June 4	–6	June 11	–1	June 4	–13	June 11	–14	–9.0
1922	June 10	+4	June 10	0	June 3	–9	June 24	+7	July 8	+13	+3.25
1923	June 9	+3	June 23	+13	June 30	+18	July 7	+20	June 30	+5	+10.75
1925	June 20*	+10.5	June 20*	+6.5	June 20*	+4.5	July 4*	+13.5	July 4*	+5.5	+8.5
1926	May 22	–15	May 29	–12	.....	.....	June 5	–12	.....	.....	–6.5
1927	June 11	+5	June 11	+1	June 11	–1	.....	.....	July 2	+7	+4.0
1928	June 16	+10	June 16	+6	June 16	+4	July 7*	+16.5	July 7*	+8.5	+5.25
1929	June 1*	–8.5	June 22†	.....	.....	.....	.....	.....	June 22†	.....	–2.5

* Record made at end of a fortnight.
† Record made at end of three weeks.

It must be noted that in 1926 there is indicated (fig. 8, S2c) a diversity in the behavior of the different species. With the available data it is not desirable to attempt to account for these, although the explanation offered in the case of *Cytisus* might be considered, namely, that a continuous advance of development through all the stages of spore formation exposes the plant for a longer time to action by external factors; and further, that at any "moment" in time the process will be more or less reactive to "momentary" fluctuations of the external factor. By momentary here is meant rather a short period, of one or two days at most.

Discussion

The foregoing data seem to indicate that the species of *Rhododendron, Cytisus,* and *Syringa* under consideration are affected as regards their date of flowering by the temperature at certain specified times during the year. While one species may in any particular year deviate from the general behavior of the natural group into which it falls here, nevertheless when all species are considered they show over a period of ten years a significantly consistent response to the temperature of distal and proximal, or proximal and immediate, weather belts.

It would seem that lower temperatures, obtaining during these significant though sometimes remote periods, delay the opening of flower buds; and conversely higher temperatures obtaining during the same periods in other years induce earlier flowering. The comparatively narrow belts of time during which deviations of temperature may be significant in inducing these effects are believed to synchronize with activity in spore formation in the plant. In other words, higher temperatures, occurring when there is activity in spore formation, lead to earlier flower burst; and lower temperatures, occurring at the same point, lead to later flower burst. The detailed mechanism through which such an effect may be brought about is deep seated and obscure. It would seem adequate simply to regard the higher temperatures as expediting the reactions involved in meiotic and premeiotic cell division; and there is no doubt that this must in large part be true.

It is a matter of common observation and frequent record that different species of plants develop generally in definitely different temperature ranges, some plants requiring for normal growth an environment with a higher temperature range than others. Again some plants are more particular and demand a narrower range of temperature; and exposure to temperature below a minimum, higher than the minimum of other more "hardy" species, inhibits development and even causes death. These specific demands are, as has been said, well known although not well understood, and may be taken as the factor which conditions the "average" flowering date of different species.

Furthermore, the temperature demands of different organs of any one plant are not the same. In the great majority of cases in the annual history of the flowering plant vegetative activity occurs first in time, and usually at temperatures lower than will occur subsequently. The vegetative activity is followed by the change to reproductive phase, the change of phase being synchronized with onset of higher temperatures, so that in a scale of rising temperatures the reproductive phase comes as a culmination to the activity of the plant. Reverse cases are known, as for example *Tussilago farfara,* in which the reproductive phase is synchronized with the earlier period of lower temperatures, and flowering precedes vegetative activity.

It may be that in the onset of the reproductive phase in such a genus as *Rhododendron,* where microspore and megaspore development take place in summer and spring respectively, this activity is dependent on temperature of narrow range, and may be inhibited, especially in the earlier flowering species, by higher as well as lower temperatures.

While stress has been laid here on the connection between temperature belts and spore formation, it is not overlooked that the metabolic complex culminating in general development may be the fundamental cause of the fluctuations. It is significant that the localization in time has been related to a definite step in development.

Summary

Analyses of records on the date of flowering of various members of different genera at the Royal Botanic Garden, Edinburgh, show the following points:

1. The actual date of flowering of any species in any one year may vary from the average date.

2. Such aberration is referable to the temperatures obtaining during narrow belts of time.

3. These belts of time vary between genera as to the remoteness from the actual date of flowering, and here are referred to the periods of activity in gamete formation.

The writer is greatly indebted to Dr. ALEX. NELSON, Edinburgh University, for much help and encouragement during the course of the work, and to Professor Sir W. WRIGHT SMITH and the staff of the Royal Botanic Garden, Edinburgh, for permission to use the data, and for other facilities.

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STUDIES IN THE GERMINATION OF

AIRA FLEXUOSA

I. MATERIAL and METHODS

Except where otherwise stated, the seed used was gathered in Bute during August and September 1931. It was allowed to dry in light until the middle of October, and thereafter stored in seed-packets until used. When the seeds were cleaned and counted for the germination tests, a large percentage of empty glumes was found. In a few cases seed from a commercial source was employed.

The germination tests were carried out in glass topped germination tanks, electrically heated. The pads on which the seeds were germinated consisted of three layers of thick blotting paper kept moist by a wick dipping into the water in the foot of the tank, and covered by a glass dome with a small hole near the apex to allow free entrance of air.

Two complete germination tests were carried out, one in February 1932 and one in November 1932. Each test comprised 32 pads, each holding 50 seeds. Eight different combinations of factors were investigated, four pads being subjected to each set of conditions. All the combinations of the following pairs of factors were employed, (a) light and dark, (b) constant temperature and fluctuating temperature, (c) tap water and a 0·2% aqueous solution of potassium nitrate.

In/

In the case of seeds germinated in the dark the glass domes over the pads were covered with light-proof paper. Where fluctuating temperature was one of the conditions the pads were transferred between one tank at 20°C and one at 30°C, being left for 8 hours at 30°C and 16 hours at 20°C. The potassium nitrate solution was supplied to the pads by dropping the ends of the wicks into small bottles of the solution standing in the foot of the tanks. All seeds were transferred to fresh pads twice during the February test and once during the November test. The tests were continued until no more seeds germinated. The first test ran from 9th till 27th February, and the second from 10th till 30th November

II. EXPERIMENTAL RESULTS

A. The Effect of After-Ripening

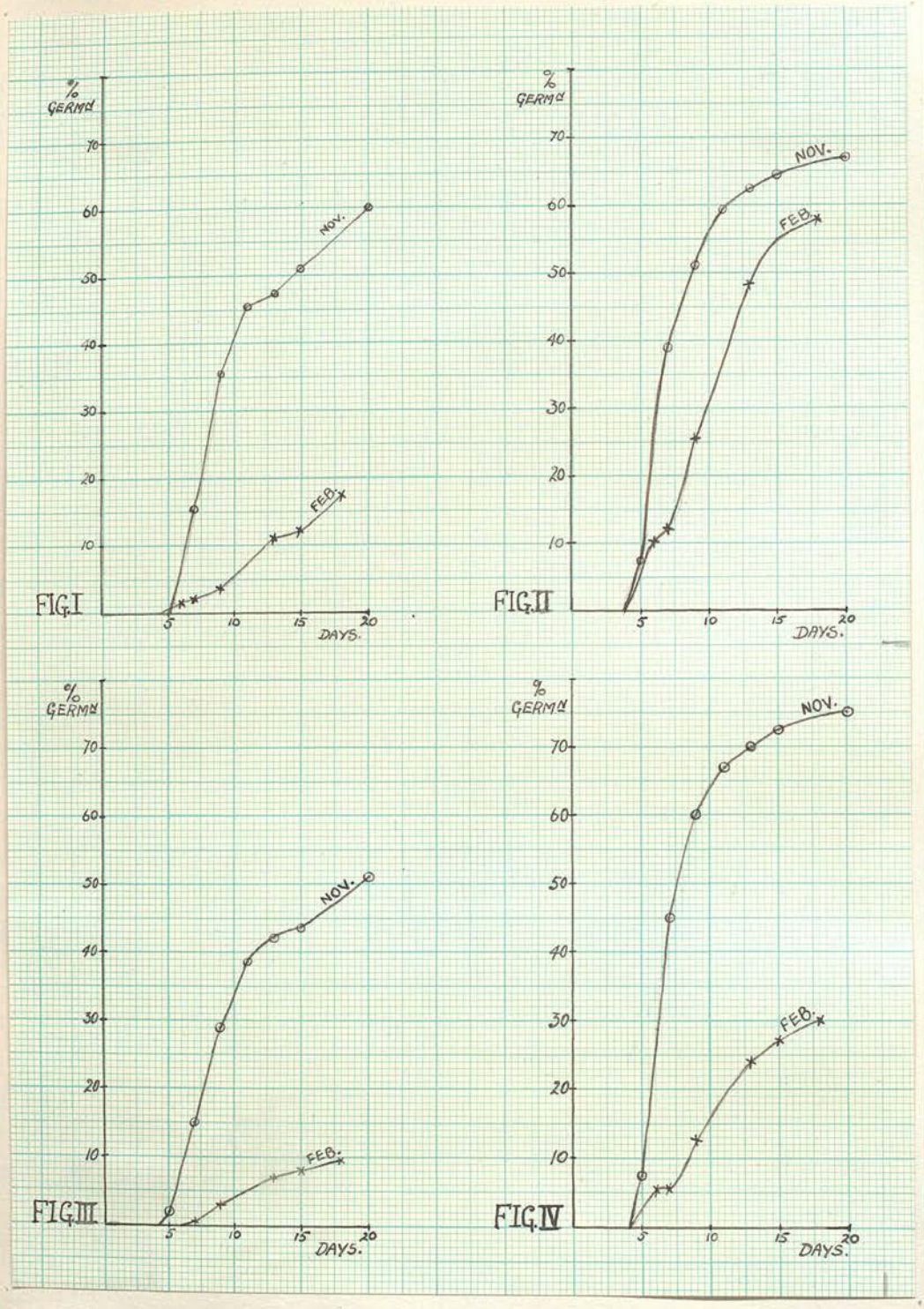
A comparison of the percentage germination in February and November shows that for each comparable set of conditions a much better percentage germination was obtained in November. This is exemplified in Table I and Figures I - VIII. The percentages are calculated from the average of four pads of 50 seeds which were under the same germination conditions.

Table I shows that the increase in percentage germination varies from 9 to 44·5 according to the germination conditions, while the average increase for all conditions is 35·3. The effect of after-ripening on germination under each combination of conditions is shown by graphs (Fig. I - VIII). The relative effects of after-ripening on germination under different conditions will be dealt with later.

TABLE/

TABLE I

Germination Conditions	% Germ Feb. 1932	% Germ Nov. 1932	Increase in Germ %
Light, Water Fluct. temp.	17·5	60·0	42·5
Light, water Const. temp.	58·0	67·0	9·0
Dark, water Fluct. temp.	9·5	51·0	41·5
Dark, Water Const. temp.	30·5	75·0	44·5
Light, Nitrate Fluct. temp.	13·5	57·0	43·5
Light, Nitrate Const. temp.	45·0	68·0	23·0
Dark, Nitrate Fluct. temp.	8·0	51·0	43·0
Dark, Nitrate Const. temp.	31·0	66·5	35·5
Average	26·6	61·9	35·3



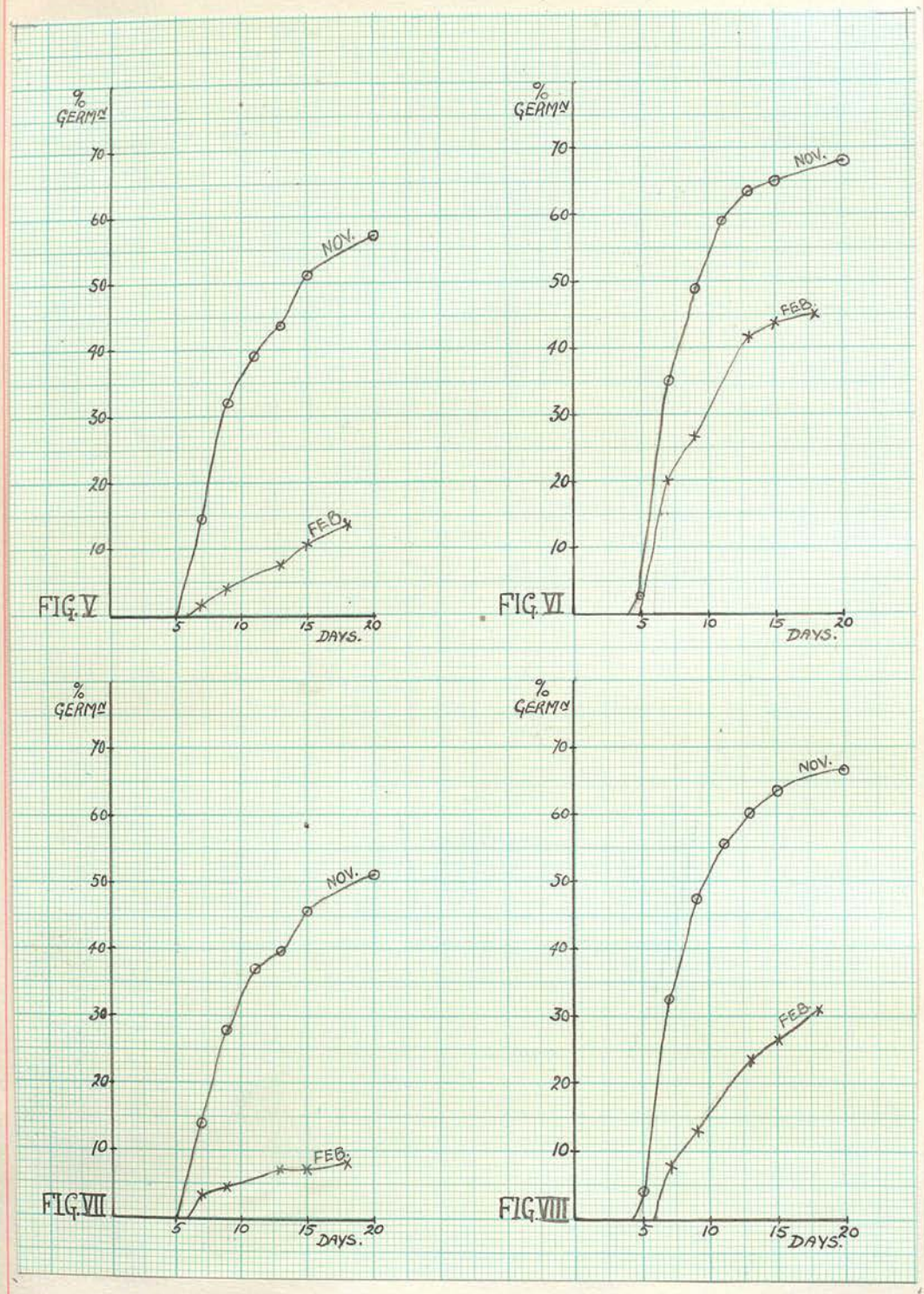
Germination of Aira flexuosa

Fig. I. Light, water, fluctuating temperature

Fig. II. Light, water, constant temperature

Fig. III. Dark, water, fluctuating temperature

Fig. IV. Dark, water, constant temperature.



Germination of Aira flexuosa

Fig. V. Light, nitrate, fluctuating temperature

Fig. VI. Light, nitrate, constant temperature

Fig. VII. Dark, nitrate, fluctuating temperature

Fig. VIII. Dark, nitrate, constant temperature.

B. The Effect of Temperature

As is shown in Table II Aira flexuosa germinates considerably better at a constant temper-:ature of 20°C than it does in an alternation of 20°C for 16 hours, followed by 30°C for 8 hours. Figure IX illustrates the course of germination in light, water and fluctuating temperature, as compared with light, water, and constant temperature. It will be observed that germination in constant temperature is more than three times the germination in fluctuating temperature. The curves for germination in dark and nitrate if drawn would show similar features. The November germinations follow the same course as the February ones, but the differences in germination under constant and fluctuating temperature are less marked. This will be discussed later, in paragaraph E. of this section.

TABLE II

Germination Conditions	February		November	
	Const. Temp.	Fluct. Temp.	Const. Temp.	Fluct. Temp.
Light, Water	58·0	17·5	67·0	60·0
Dark, Water	30·5	9·5	75·0	51·0
Light, Nitrate	45·0	13·5	68·0	57·0
Dark, Nitrate	31·0	8·0	66·5	51·0

FIG IX

Germination of Aira flexuosa

Fig. IX. February 1932

- - - Light, water, fluctuating temp.

——— Light, water, constant temp.

C. The Effect of Light

The figures for February in Table III show that Aira flexuosa germinates better in light than in dark. Figure X illustrates this point with the curves of germination in light and dark under similar conditions of water and constant temperature. As with temperature the difference is not nearly so marked in the November germinations. Indeed in one case better germination is obtained in the dark.

TABLE III.

Germination Conditions	February		November	
	Light	Dark	Light	Dark
Water, Const. temp.	58·0	30·5	67·0	75·0
Water, Fluct. temp.	17·5	9·5	60·0	51·0
Nitrate, Const. temp.	45·0	31·0	68·0	66·5
Nitrate, Fluct. temp.	13·5	8·0	57·0	51·0

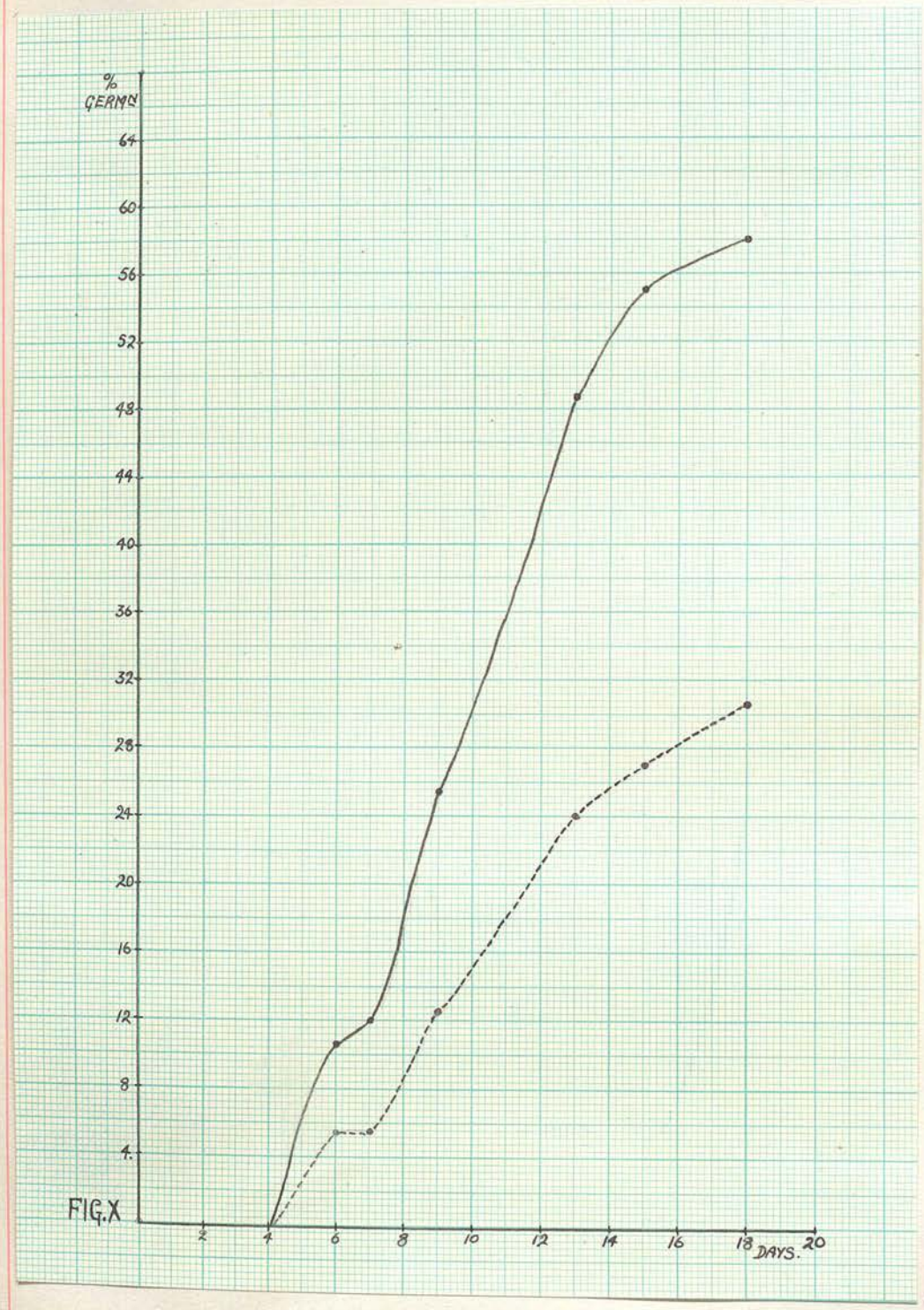


FIG.X

Germination of Aira flexuosa

Fig. X. February 1932

- - - Dark, water, constant temp.

——— Light, water, constant temp.

D. The Effect of Potassium Nitrate and Acids.

Apparently a 0·2 % solution of potassium nitrate has no effect on the germination of Aira flexuosa. Table IV. shows slight variations from 1% better germination in nitrate to 13% better germination in water, with a mean difference in percentage germination of 3·5, in favour of water. This can not be regarded as significant.

TABLE IV.

Germination Conditions	February		November	
	Water	0·2% KNO_3	Water	0·2% KNO_3
Light, const. temp.	58·0	45·0	67·0	68·0
Light, fluct. temp.	17·5	13·5	60·0	57·0
Dark, const. temp.	30·5	31·0	75·0	66·5
Dark, fluct. temp.	9·5	8·0	51·0	51·0

The question of the effect of substrate on the germination of Aira flexuosa was further investigated using solutions ofpotassium mitrate, nitric acid, sulphuric acid, and hydrochloric acid of various concentrations. Commercial seed was employed in these experiments which were all carried out at 20°C in light. The first test (Table V) was run for 10 days, starting on 2nd December 1932, and the second test (Table VI) was/

was run for 10 days, starting on 14th March 1933. The figures show that sulphuric and hydrochloric acids in all the concentrations employed (0·1 M, 0·01 M, 0·001 M) retard germination. Nitric acid and nitrate on the other hand are harmful only in stronger concentrations, and in weaker concentrations have no appreciable effect on germination, either retarding or stimulating.

TABLE V.

Germination Conditions	% Germ
Tap water	44·5
0·05M KNO_3	47·0
0·1M KNO_3	15·0
0·1M HNO_3	0·
0·1M H_2SO_4	0·
0·1M H Cl	4·0

TABLE VI.

Germination Conditions	% Germ
Tap water	40·0
0·01M KNO_3	29·0
0·001M KNO_3	35·0
0·01M HNO_3	22·0
0·001M HNO_3	43·0
0·01M H Cl	26:0
0 001M H Cl	19·0

E. The Relationship of After-Ripening to the other Factors affecting Germination.

In the consideration of the various factors affecting germination it has been noted in passing that the percentage germinations under different conditions in November show much smaller differences than do the percentage germinations under different conditions in February of the same year. If the factors are considered in pairs, i.e. light and dark, fluctuating and constant temperature, where one of a pair has a depressing effect on the germin-:ation of un-after-ripened seed the increase in per-:centage germination as after-ripening proceeds is much greater for seeds germinated in the presence of that factor than it is for seeds germinated under the influence of the other member of the factor pair. For instance the average increase in germination due to after-ripening is 42·6% in the case of fluctuating temperature, and only 28·5% in the case of constant temperature, although constant temperature is the more favourable condition for germination. In the same way there is an average increase of 41% in dark and 29·5% in light. Moreover, the greater the influence of a factor on the germination of un-after-ripened seed, the less is the increase on after-ripening in germination of seeds under the influence of/

of this factor, relative to the increase in germin-:ation of seeds under the influence of the other member of the factor pair. In the February tests (i.e. un-after-ripened seed) constant temperature gave an average of 29% better germination than fluctuating temperature, light an average of 13·75% better germination than dark, and water an average of 4·5% better germination than potassium nitrate. If the differences in increase in percentage germin-:ation on after-ripening are calculated, it is found that they occur in the same order.

	Increase in % Germ	Difference
Const. temp.	28·5	14·1
Fluct. temp.	42·6	
Light	29·5	11·6
Dark	41·1	
Water	34·4	1·6
Nitrate	36·0	

Figs. XI and XII contrast the germination under the most favourable combination of conditions, i.e. light, water, and constant temperature, with germination under the least favourable combination i.e. dark, nitrate, and fluctuating temperature. From these it can be seen how relatively large is the/

the effect of after-ripening on germination in un-
:favourable conditions.

As after-ripening proceeds the factors which influence germination of the un-after-ripened seed become less important, so that germination of after-ripened seed is much nearer constancy under varying conditions than is the germination of un-after-ripened seed.

Germination of Aira flexuosa

Fig. XI. Light, water, constant temp.

--- February 1932

—— November 1932

Germination of Aira flexuosa

Fig. XII. Dark, nitrate, fluctuating temp.

- - - February 1932

——— November 1932

III. DISCUSSION

The factors affecting the germination of seeds have been the subject of a considerable amount of research. This is particularly so in regard to light, which has been studied, especially by German workers, in great detail. These studies have been concerned not only with light as the only variable but also with light in connection with other factors, such as temperature and substrate. There is much conflict of opinion as to the mode of action of light. Temperature, temperature fluctuations, the use of acids and salts in various concentrations, after-ripening, harvesting, and storage have received attention from German workers largely in respect to their interactions with light, but temperature and after-ripening at least have also been given more individual attention by certain American workers.

1. Temperature

Seeds of different species, and frequently seeds of the same species from different sources, vary considerably in their temperature requirements. Doerfel (1930) records that for certain species germinated at constant temperatures the best results are obtained at 0°C and 5°C. For most species the optimum/

optimum temperature is appreciably higher, being in general between 15°C and 30°C. Edwards (1932) gives a comprehensive account of the maximum, minimum, and optimum germination temperatures of various species, so far as they are known. The optimum temperature for germination may be influenced by other factors. For instance Harrington (1923') and Munerati (1925) have found for wheat, and Atterberg (1928) for barley, that freshly harvested grain has a lower temperature optimum than grain which has been stored. On the other hand Gassner (1930) reports for Poa that although at constant temperature germination is much better in after-ripened than in un-after-ripened seeds, the optimum temperature in both cases is 12°C. Ten species of Labiatae and Cruciferae have been found to have the same temperature optimum in light as in dark (Bihlmeier 1929). It is quite possible however that this may not apply in all cases.

The seeds of a large number of species show considerably better germination in regular alternating temperatures than they do in constant temperature. Thus Harrington (1923[2]) states that redtop (Agrostis spp) parsnip, and some Petunia seeds give somewhat better, and celery, orchard grass, Kentucky blue-grass, Bermuda grass, and Johnson grass much better germination with certain alternating temperatures than with constant temperatures/

temperatures. On the other hand there is no such increase of germination in the case of carrot, parsley, timothy, awnless brome grass, perennial and Italian ryegrass, and meadow fescue. The best results are obtained when the higher of the two temperatures is maintained for only a small part of the day, never more than 8 hours and generally less, and the change to the lower temperature is rapid. It is also shown that different lots of the same kind of seed vary in their temperature sensitiveness. This is probably the effect of differences in degree of after-ripening, or conditions of harvesting and storage. In the case of Johnson grass Harrington observes that the best germinating lots of seeds are also the least exacting as to temperature conditions.

Gassner (1911) working with Chloris ciliata has also found alternating temperatures to be most beneficial when the higher temperature is applied for the shorter and the lower for the longer daily period. Indeed with the reverse arrangement germin-
:ation of this species is little better than in con-
:stant temperatures. When however the glume is re-
:moved from the grain there is an increase in ger-
:mination which is not heightened further by conditions of intermittent temperature.

Morinaga (1926) has found like Harrington that/

that some species show improved germination in alter-:nating temperatures, while others germinate just as well at favourable constant temperatures. He also shows that the alternation most effective for one species is not necessarily the most effective for another. Alternation was found not to be effective if the daily duration of either the higher or the lower temperature was less than 4·5 to 8 hours. It may be noted in passing that other workers have obtained satisfactory results when one of the temperatures has been applied for 4 hours daily. Mechanical injury to the seed-coat or treatment with sulphuric acid produces good germination of Cynodon dactylon and Typha latifolia at constant temperature, while light and nitrate give increased germination of Poa compressa at constant temperature, although the highest percentage germin-:ation is only obtained when alternating temperature is applied in conjunction with light and nitrate.

In a recent paper on certain species of Poa Gassner (1930) has found that a single temperature fluctuation, either from warm to cold or cold to warm, gives better germination than constant temper-:ature, provided that the length of exposure to the first temperature is neither too short nor too long, the actual optimum exposure depending on the temper-:ature. He attributes the harmful effect of too long an/

an exposure in the first temperature to a "Hemmungsvorgang" "Die Versuchsergebnisse lassen sich dahin deuten, dass "jeder längere Aufenthalt bei einer konstanten Temper- ":atur zu einer Selbsthemmung des Keimungsvorganges "führt; diese Hemmungsvorgänge sint offenbar temper- ":aturspezifisch, da sie sich durch Anwendung einer "abweichenden Temperatur unterbrechen lassen". Here again it has been found that the best results with alternating temperature are obtained if the higher temperature acts for the shorter time. But this, although generally true, is not always the case. Gassner (1915') reports the reverse to be true for Oenothera biennis, and Doerfel (1930) observes that for Hyoscyamus germination is better if the higher temperature acts for the longer time. It is also necessary for this species that the temperature interval should be sufficiently large. The small variation 25°/ 30° gives lower germination than constant temperature.

Several suggestions have been put forward as to why germination should be better in alternating than in constant temperature. Liebenberg (1884) has suggested that at any given temperature the available reserve materials are largely used up in respiration, but if the temperature is suddenly lowered the surplus immediately on hand can be used for growth. This is either an incorrect or an incomplete explanation in view/

view of the fact that a single alternation from cold to warm has been found to give better germination than constant temperature. Harrington (1923[2]) suggests almost the converse of this, namely that at low temperatures there is an accumulation and metabolism of oxygen in a form which becomes immediately available for growth at the onset of the higher temperature. This is also invalidated as a general explanation since a single temperature change from warm to cold causes increased germination. Vanha (1899) points out that differences in temperature between different parts of the seed, the germinating bed, and the air immediately surrounding the seeds, following a rapid temperature change would be liable to cause different gas densities which might set up gas movements leading to the removal of carbon dioxide and renewal of oxygen, conditions favourable for respiration, and presumably for germination. On this theory also doubt is thrown by the fact that a single temperature change is effective in causing a very considerable increase in germination.

When Gassner (1911) found that removal of the glumes of Chloris ciliata brought about good germination at constant temperatures and moreover that the glumeless grains were no longer susceptible to intermittent temperature, he concluded that intermittent temperature/

temperature acted more or less by annulling the glume function and allowing free ingress of oxygen. "Für Chloris "ciliata halte ich den Nachweis für erbracht, dass die "keimungsfördernde Wirkung der intermittierenden Temper- ":aturen nicht, wei bisher meistens angenommen, in einer "Reizwirkung, sondern in einer Verbesserung der Sauer- ":stoffverhältnisse bei gleichzeitig möglicher Amvendung "hoher Keimungstemperaturen besteht. Die Wirksamkeit "der intermittierenden Behandlung zeigt sich an die "Existenz einer sauerstoffzutritterschwerenden Schicht "gebunden, als welche bei den Scheinfrüchten von Chloris "ciliata die Spelzen wirksam sind".

In his more recent work on Poa Gassner (1930) finds that grainsfrom which the glumes have been removed are still susceptible to alternation of temper- :atures, so that in this case at least increase of oxygen cannot be the mode of action of the temperature change. In view of the fact already mentioned, that too long an exposure to one temperature, before alternation to another, proves harmful, he considers that regulated intermittent temperatures give increased germination not because they are directly beneficial, but because they allow evasion or overtaking of temperature-specific hindrance effects "Wenn regelmässig intermittierende "Temperaturen die Keimung von Poa-Samen im Gegensatz "zur Einwirkung dauernd konstanter Temperaturen "ermöglichen, also nicht eigentlich "auslosen", so " liegt das daran, dass durch die Kombination "einer tiefen und einer hohen "Temperatur"

Temperatur im richtigen Zeitverhältnis (bei 12°: 28° ca. 7 Zeiteinheiten 12° auf 1 Zeitenheit 28°) die Ausbildung der Temperatur spezifischen Hemmungsvorgänge vermieden oder überholt wird."

Doerfel considers that intermittent temperatures probably operate on certain physiological processes, the connection of which with certain temperature points would explain the marked superiority of some temperatures and temperature combinations.

2. Light

As regards light seeds fall into three classes (1) light aided (2) light-indifferent (3) light-hindered. Depending on other factors different samples of the same species may belong to different groups or at least vary in their degree of light sensitiveness. Lehmann (1912) has reported that different samples of Epilobium roseum exhibit different degrees of light requirement, hence it is necessary to work with a pure line to obtain reliable results. In the case of Verbascum thapsiforme seeds gathered from the under part of the inflorescence gave only 10% less germination in the dark than in the light in ten days, while if the seeds were gathered from the upper part of the inflorescence germination was 40% less in the dark. Maier (1932) notes that harvesting time affects the light sensitiveness of Poa nemoralis/

nemoralis. The most decisive factor in light sensit-:iveness is perhaps degree of after-ripening. Johnsson (1883) has shown that seeds of Poa pratensis in the un-after-ripened condition germinate up to 88% in the dark and not at all in the light, while after twelve months they germinate equally well in either. Gassner (1911) finds that the un-after-ripened grain of Chloris ciliata requires light while the after-ripened does not. The same writer later reports (1915) that after-ripening brings about progressive alter-:ation in the seeds, so that they are at first aided by light, next indifferent to it, and finally hindered by it as regards their germination. Germination temperature comes into the question inasmuch as with low temperatures the inhibiting action of light appears earlier in after-ripening than it does at higher temperatures, so that with seeds at a medium stage of after-ripening light is hindering at low temperatures and stimulating at high temperatures.

Busse (1926) using a number of varieties of Tobacco, which are mainly light germinators, finds that germination itself is not affected by darkness, but only the time required for it to take place. Wieser (1927) finds that the seed of Lythrum salicaria which can germinate up to 50% in the dark, show decreased ability to germinate in the dark with age. Kinzel (1917) believes that light and dark play an important/

important part in the germination of seeds in nature, and that different reactions in this respect are to be regarded as a response to environmental conditions. The seeds of dune plants are subject in nature to deep burial, and they generally germinate better in dark than in light. On the other hand the seeds of plants inhabiting firm ground are usually hindered by dark. It is quite possible that this view may be correct but until more data is available it cannot be generally applied.

Maier (1932) has found that Poa species, par-:ticularly Poa nemoralis, are sensitive to light and give better germination under its influence. This light sensitivity increases after the seeds are put to germinate, and, after reaching a maximum, falls away again. The point of highest light sensitiveness depends on temperature. Parallel with this there is a variation in sensitiveness to temperature fluctuation. Maier considers that the term "Lichtempfindlichkeit" (Light sensitiveness) should be replaced in certain cases by the term "Lichtbedürfnis" (Light-requirement) and that the statement that light sensitiveness decreases with after-ripening should be amended in thisrespect. In other words it is light-requirement which decreases in the course of after-ripening.

As a general rule it has been found that temperature conditions have a profoundly modifying effect/

effect on the action of light. Lehmann (1912) observes that within the temperature limits that allow of germination, with the light germinating seeds examined (Epilobium roseum, E. hirsutum, E. palustre, Veronica longifolia), the higher temperatures encourage germination in the dark, while with the dark-germinating seeds (Phlox Drummondii, Nemophila insignis Whitlavia grandiflora) the lower temperatures promote germination in the light. Fluctuating temperature has an even more marked influence on the light action. Gassner (1915[1]; 1915[2]) demonstrates this in the case of Ranunculus sceleratus. This species shows negligible germination in both dark and light in both high and low constant temperatures. With suitable temperature alternation however it germinates in either light or dark. In constant dark the best results are obtained with a large temperature interval (14°C) and the higher temperature applied for the shorter time. No data is given for germination in constant light, but when the seeds are exposed to day-light for part of the day the germination percentages are higher and the necessary temperature interval smaller. As no experiment was carried out using fluctuating temperature and constant daylight it is not known whether light or alternation of light and dark causes the increase in germination in this instance. Fassbender (1925) states that intermittent illumination of/

of the seeds of Epilobium hirsutum has a greater effect than constant light, while Lakshmano Rao (1925) using Lythrum salicaria finds intermittent light to have the same effect as the sum of the light intervals. Gassner (1915[2]) next turns his attention to the question of whether light fails to act as an aid to germination at constant temperatures because it is ineffectual at constant temperatures, or because, though the seeds are altered in some way by the light, they fail to germinate because of the need for temper-:ature fluctuation. Since he finds that seeds illumin-:ated at constant temperature and then transferred to darkness and fluctuating temperature give a higher percentage germination than seeds germinated in darkness and fluctuating temperature without previous exposure to light, he concludes that light does exercise some effect on the seeds at constant temper-:ature, an effect which remains latent until the seeds are presented to suitable temperature conditions.

Fluctuating temperature however does not always enable light-sensitive seeds to germinate in the dark. Hutchings (1932) reports that the seeds of Mimulus ringens do not germinate in the dark either with high or low constant temperatures or with alternation of temperature. In diffuse light high constant temperatures cause an increase in germination, but there is no data on the effect of fluctuating tem-:perature in diffuse light.

Light/

Light-sensitive seeds appear to be particularly sensitive also to the acidity of the substrate. Ottenwälder (1914) has establised that eight species which otherwise require light for germination will germinate in the dark at suitable temperatures if provided with an acid substrate, while two other species closely related to several of these eight, but differing from them in that they are not light-sensitive, fail to receive any benefit from similar concentrations of acid. Gassner (1915[2]) has followed this up with a fairly wide survey of the effects of chemical substances, both germination-aiding and otherwise, in relation to light. Knops solution at the most favourable temperature combinations enables Ranunculus sceleratus to germinate as well in dark as in light. Of the other chemical substances it may be said in general that while weak solutions may or may not aid germination according to their chemical nature, strong solutions have a deleterious effect .

Both Kuhn (1916) and Lehmann (1919) have found that light-hindered seeds are affected by substrate. The former has shown that suitable concentrations of hydrochloric, sulphuric, or nitric acid enable the seeds of Phacelia tenacetifolia to germinate in light. The actions of dark and acid are however not additive since the same concentration of acid which produces germination in light does not give increased but rather poorer/

poorer germination in dark. Lehmann reports that the light-hindered seeds of Veronica Tournefortii are favourably affected by nitrate.

Nelson (1927) using several species of Poa has observed that although potassium nitrate and other salts were stimulating in culture they had a depressant effect when the seeds were germinated in soil. Maier (1932) also reports nitrates and to some extent hydrochloric acid as aiding the germination of Poa.

Light-requirement can sometimes be altered by the removal of glumes or testas. Chloris ciliata with glumes requires light, but is indifferent to it after their removal (Gassner 1911), while the removal of the testa of the light-hindered Phacelia tenaceti-:folia in the same way dispenses with the necessity for dark (Böhmer 1928). In both cases the effect is attributed to the increased supply of oxygen reaching the seed. This is not necessarily the case. The two light-hindered species Phacelia tenacetifolia and Nigella sativa can germinate in light if the oxygen content of the medium is increased, but the removal of the seed-coat of Nigella sativa does not enable it to germinate in light. Axentjev (1929) considers that seed-coats may hinder germination by restricting the supply of oxygen to the seed. It is at least evident that light does not always act through the medium of oxygen/

oxygen intake since Wieser (1927) has found that Lythrum salicaria can germinate in light in the complete absence of oxygen.

A few workers have carried out investigations in connection with the amount of light necessary to promote the germination of light-requiring seeds. In the case of Lythrum salicaria Lehmann (1918) finds that using 730 c.p. there is a perceptible effect with illumination of 1 minute at 20°C, or 1/10 second at 30°C., and Wieser (1927) using 200 c.p. gets a comparable effect in 1 minute while the maximum effect is usually reached with about 12 hours illumination. Hutchings (1932) finds that much longer exposure to light is necessary in the case of Mimulus ringens. Using continuous illumination with light of different intensities Ottenwälder (1914) has shown for a sample of Epilobium that the minimum effective light-strength is 3 to 0·5 c.p. at 20°C, while at 25°C $\frac{1}{400}$ c.p. does not reach the lower limit. As might be expected the light-requirements of different species, and even different samples of the same species, are by no means uniform. Lakshmano Rao (1925) finds that the percentage germination of Lythrum salicaria at 31°C and 35°C is directly proportional to the amount of light received, within a certain range. Above this range the position is reversed. Kommerell (1927) proves for the same species that the effect of light

on/

on germination is directly proportional to the wave length.

There are a number of rival theories as to the mode of action of light in promoting germination. Lehmann (1912) and Lakshmana Rao consider that in the case of Lythrum salicaria and Epilobium hirsutum light acts as a stimulus. This is argued on the basis of certain similarities which appear to exist between the light action on these seeds and the behaviour of light acting as a stimulus on other plant organs. In another paper (1913) written in conjunction with Ottenwälder, Lehmann declares that light has a catalytic effect, and offers the three hypotheses (1) that it hastens and increases enzyme action (2) that it activates zymogen present in the resting seed (3) that it itself acts as a catalyser in the presence of certain substances. Ottenwälder (1914) finds that light can be replaced by acid, and considers that both have a catalytic action. Gassner (1915) is opposed to the idea of light as a stimulus on account of the fact that light requiring seeds, given a sufficient exposure to light in temperature conditions unsuited to germin-:ation and then subjected to drying, subsequently germinate given suitable temperature, in the dark. He considers that an inhibiting factor operates during germination, whose action is hindered by light. Finally Kommerell believes light to have a photochemical action.

3/

3. After-Ripening

The seeds of some species will not germinate immediately after they are ripened and shed from the plant. This may be due to "hardness", mechanical resistance of the seed-coat, reduction of oxygen by the seed-coat, morphological immaturity of the embryo, or it may be due to the need for a period of after-ripening. The changes which take place during this process are as yet imperfectly understood, but alterations in acidity, enzymes, and food-reserves have been observed. After-ripening seems to occur most rapidly at comparatively low temperatures (0^{o}- 5^{o}C) This is reported by Pack (1921) for Juniperus, by Davis (1927) for Cornus florida and Sambucus canademsis, by Joseph (1929) for Betula, and by Flemion (1933) for Rhodatypos Kerroides. As Joseph points out this would enable seeds to after-ripen on the ground during winter.

As regards changes in acidity Eckerson (1913) has found that the dormancy of the Crataegus embryo is due to its dormant hypocotyl, and that this organ, at first slightly alkaline or neutral, becomes distinctly acid with after-ripening. Rose (1919) has shown that a slight increase in acidity accompanies the after-ripening of Tilia seeds. In both, increased acidity is accompanied by increased water-holding power, of the hypocotyl in the former, and the embryo in the latter. Jones shows that in the sugar maple the

embryo/

embryo is always basic, although the hydrogen ion concentration may increase in the embryo as it after-ripens, and that there is no rise in the water holding capacity of the seeds. Increased water-holding power is probably connected with increased acidity, owing to the hydrophilous colloids having a greater water-holding capacity in an acid medium.

As regards enzyme activity Eckerson (1913) Rose (1919), Jones (1920) and Davis (1927) all find a rise in catalase activity as after-ripening progresses, while Crocker and Harrington (1918) report an increase in the activity of both catalase and oxidase.

The chemical changes which accompany after-ripening in general seem to involve an increase in carbohydrate and a decrease in stored protein. Jones reports that after-ripening in sugar-maple is accompanied by an increase in the amount of free reducing sugars, and the same is found by Okada (1930) for Euryale Ferox. Pack finds a decrease in the amount of stored fat and Protein, with increase in sugar content, and first appearance of starch in the case of Juniperus. He also finds an enormous increase in the degree of dispersion of stored fat, translocation of food in the form of fats or fatty acids from the endosperm to the embryo, increase in soluble proteins and marked hydrolysis of stored proteins, a seven-fold increase in amino acid content, and complete disappearance of histidine. Davis observes in the case of Cornus florida an increase in starch, sugar/

sugar, and amino acids, with little or no change in fats, acidity, or phosphatides.

IV. SUMMARY AND CONCLUSIONS

It is apparent from the foregoing discussion that light sensitive seeds are also sensitive to temperature and substrate, being in general favourably affected as regards their germination by fluctuation of temperature, slight acidity of the substrate, or presence of nitrates in weak solution. Aira flexuosa, at least under the conditions of these experiments, is adversely affected by fluctuation of temperature, and practically unaffected by anid or potassium nitrate in weak solution, although it is light-sensitive.

The seeds of Aira flexuosa require a period of after-ripening for good germination, and in the after-ripened condition are less sensitive to external factors than in the un-after-ripened condition.

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PHYSIOLOGICAL STUDIES IN BRASSICA ALBA

I. THE TISSUE REACTIONS OF B. ALBA.

II. THE BUFFER SYSTEM OF B. ALBA.

III. THE EFFECT OF THE MUCILAGE OF THE SEED-COAT IN GERMINATION OF B. ALBA.

IV. THE DISTRIBUTION OF STARCH IN THE RADICLE OF B. ALBA.

THE TISSUE REACTIONS OF BRASSICA ALBA

I. MATERIAL AND METHODS

The method employed was that developed by Small (1929), known as the "Range Indicator Method," by which the p H of a tissue is determined by the reactions of a series of indicators with overlapping ranges. The reactions of all the tissues of B. alba were found to fall within the ranges of the following five indicators. Aqueous solutions were preferred to alcoholic solutions, as liable to give more accurate results. The benzene-azo-d-naphthylamine however being insoluble in water was dissolved in 30% alcohol.

Brom-cresol-purple (B.C.P.)0·04% mono Na salt

Di-ethyl red (D.E.R.)0·02%

Methyl red (M.R.) 0·02%

Benzene-azo-d-naphthylamine (B.A.N.) 0·01% hydrochloride

Bromo-cresol-green (B.C.G.)0·04% mono Na salt

The ranges and colour reactionsof these indicators are as follows.

B.C.P.	Pale blue - deep purple	> pH	6·2	yellow	< pH 5·9
D.E.R.	Yellow	>	5·9	pink-red	< 5·6
M. R.	Yellow	>	5·6	pink-red	< 5·2
B.A.N.	Yellow	>	4·8	pink-red	< 4·4
B.C.G.	Pale green-deep blue	>	4·4	yellow	< 4·0

All sectioning was carried out using freshly prepared/

prepared neutral water. This is obtained by adding tap water to distilled water until the mixture is colourless to Phenol Red.

Hand-sections of the tissue to be examined were cut, placed temporarily in neutral water, transferred to clean watch-glasses, and covered with indicator. The minimum time required for penetration of the indicators was from 2 to 3 hours and it was found that the reaction of the tissues remained the same for at least 20 hours after this; consequently the convenient procedure was adopted either of cutting the sections in the morning and examining them in the afternoon, or of cutting them in the afternoon and leaving them overnight in the indicators. Several sections of the same tissue were always stained in each indicator as a check in case of different rates of penetration due to varying thickness of the sections. The examination was always carried out in daylight, as artificial light, even with a "daylight" electric bulb was found to be unsatisfactory.

The seed was thinly sown in four inch pots on October 18th 1932 and the pots kept throughout in a greenhouse at 45-60°F. The plants to be examined were carefully removed from the pots and sectioned immediately. A description of the stages of growth reached by the plants at the time of their removal is given below. For each stage three plants at least were used, and care/

care was taken that all plants should be at similar stages of development. Where imperfect penetration of the indicators left room for doubt after the examination of three plants, further material was investigated. No data is available for the laminae of the cotyledons as the indicators entirely failed to penetrate these organs, Material of stage II (see below) was obtained by germination on filter paper in a germinating tank at 20°C. Investigation showed that the tissue reactions of seeds germinated in this way were identical with the reactions of seeds germinated on soil. For investigation of stage I the seeds were soaked in neutral water in order to facilitate sectioning. It was found that seeds soaked for forty-eight hours had the same reactions as those soaked for five minutes (just sufficient to allow of cutting) Where necessary pith soaked in neutral water was used to hold the material being sectioned.

The developmental stages investigated were :—

Stage I. Ungerminated seed.

Stage II. Germinated seed, radicle just appearing

Stage III. Seedling 4-8 days old. About 5 cms. high Cotyledons fully expanded. First foliage leaves only just visible Root 3-4 cms.

Stage IV. Four weeks old. First two foliage leaves expanded. Epicotyl about 1 cm.

Stage V. Six weeks old. Four foliage leaves expanded, no internodes yet sufficiently developed to be sectionable. Outer cortex of the root beginning to dis-:appear

Stage VI. Eight weeks old. Six leaves expanded. Epicotyl about 3 cms. Second and third internodes each about 1 cm. (First internode remains short)

Stage/

Stage VII. Fifteen weeks old. Eight leaves expanded, the first two yellow and ready to drop off: Cotyledons shed. Total height above ground about 17 cms. Lower part of stem with complete ring of xylem, and small bundles of fibre in the inner cortex.

Where a p H value appears in brackets in the following tables it signifies that there is some doubt as to the correctness of the figure on account of difficulty in determining the tint of the indicator or because of its occurring only once in the series of observations on the particular tissue, or for both reasons. Where one figure of a p H range is in brackets e.g. 5·6 — (4·8) it indicates that the bracketed end of the range given is in some doubt and may possibly be too extreme, for the reasons already given

II. EXPERIMENTAL RESULTS

Table I. Stages I & II

	Stage I	Stage II
Cotyledons	5·9 (5·6)	5·9 (5·6)
Radicle	5·9 (5·6)	5·9 (5·6)
Testa - Epid.	6·2 - 4·8	6·2 - 4·8
Sub-epid	6·2 - 4·8	6·2 - 4·8
U. Pal.	5·2 - 4·8	5·2 - 4·8
L. Pal.	(5·9) - 5·6	5·6
Aleur.Layer	5·9	5·9

The tissue reactions of the seed were somewhat difficult to determine, since many of the tissues did not stain up well in the indicators. This led to some uncertainty when dealing with those indicators which are yellow at one end of their range, as it is often extremely difficult to be sure whether a tissue is pale yellow or merely unstained. This was the case with the cotyledons and radicle to some extent, though examination of a large number of sections led to the conclusion that the p H lay between 5·9 and 5·6. In the case of the epidermis and sub-epidermis the only three indicators which could be said to give a definite yellow colouration were Phenol Red, B.T.B., and B.A.N., so the reaction of these tissues could not be determined more precisely than as p H 6·2 - 4·8. With the/

the aleurone layer another difficulty appeared. This layer has a definite yellow colour in untreated sections, and is yellow in all indicators except B.C.G. in which it is a strong blue green, and therefore definitely above pH 4·4. As the sections treated with the other indicators all showed a deepening of the colour in this layer the pH is probably 5·9. With regard to the palisade layer, this, although a single layer, gave different reactions for the upper and lower halves of the walls, the line of demarcation being fairly sharp.

TABLE II

STAGE III

TISSUES	ROOT	BASE OF HYPOCOTYL	MIDDLE OF HYPOCOTYL	TOP OF HYPOCOTYL	PETIOLE OF COTYLEDON
Pith	—	—	5·9	5·9	—
Xylem	5·2 - 4·8	5·6 - 4·8	5·6 - 4·8	5·6 - 4·8	5·6 - 4·8
Phloem	5·9	5·9	5·9	5·9	5·9
Endodermis	5·6	5·9 - 5·6	5·9 - 5·6	—	—
Cortex	5·9 - 5·6	5·9	5·9	5·9	—
Epidermis	—	—	—	—	5·6
Epid. hairs	—	—	5·6	5·6	5·6
Palisade	—	—	—	—	5·9
Mesophyll	—	—	—	—	5·9

There is no data for the lamina of the cotyledon as/

as all indicators failed to penetrate the tissues of this part of the plant. It was impossible also to determine the pH of the epidermis of the hypotcotyl due to the presence of purple colouration in the cell sap.

TABLE III. STAGE IV.

TISSUES	ROOT	BASE OF HYPOCOTYL	MIDDLE OF HYPOCOTYL	TOP OF HYPOCOTYL	EPICOTYL	PETIOLE OF COTYLEDON	PETIOLE OF 1st.LEAF	LAMINA OF 1st. LEAF
Pith	——	——	——	5·9	5·9	——	——	——
Xylem	5·2 - 4·8	5·2 - 4·8	5·6 - (4·8)	5·6	5·6	5·6 (5·2)	(5·9) 5·6	(5·9) 5·6
Phloem	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9
Endodermis	5· 6	5· 6	5·9 - (5·6)	——	——	——	——	——
Cortex	5· 9	5· 9	5· 9	5·9	5·9	——	——	——
Epidermis	——	5·6	5·6	5·6	5·6	5·6	5·6	5·6
Epid. hairs	——	5·6	5·6	5·6	5·6	5·6	5·6	5·6
Palisade	——	——	——	——	——	——	——	5·9
Mesophyll	——	——	——	——	——	5·9	5·9	5·9

STAGE V.

TISSUES	ROOT	BASE OF HYPOCOT.	MID. OF HYPOCOT.	TOP OF HYPOCOT.	EPICOTYL	PETIOLE OF COTYL.	PETIOLE 1st. LEAF	LAMINA 1st. LEAF	PETIOLE 3rd. LEAF	LAMINA 3rd. LEAF
Pith	—	—	—	—	5·9	—	—	—	—	—
Xylem	5·2–4·8	5·2–4·8	5·6–4·8	5·6	5·6	(5·9) 5·6	5·6	5·6	5·6 (5·2)	5·6
Phloem	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9
Endodermis	—	5·6	5·9	—	—	—	—	—	—	—
Cortex	—	5·9	5·9	5·9	5·9	—	—	—	—	—
Epidermis	—	5·6?	5·6	5·6	5·6	5·6	5·6 (5·2)	5·6	5·6	5·6
Epid. hairs	—	5·6	5·6	5·6	5·6	5·6	5·6 (5·2)	5·6	5·6	5·6 (5·2)
Palisade	—	—	—	—	—	—	—	5·9	—	5·9
Mesophyll	—	—	—	—	—	5·9	5·9	5·9	5·9	5·9
Pilif.layer	5·6–4·8	—	—	—	—	—	—	—	—	—

TABLE V. STAGE VI.

TISSUES	ROOT	BASE HYPOCOT.	MIDDLE HYPOCOT.	TOP HYPOCOT.	EPICOT.	2nd INT.	PETIOLE COTYLEDON	PETIOLE 1st LEAF	1st LEAF	PETIOLE 5th LEAF	5th LEAF
Pith	—	—	—	—	5·9	5·9	—	—	—	—	—
Xylem	5·2-4·8	5·2-4·8	5·2-4·8	5·2-4·8	5·6	5·6	5·6	5·6	5·6	5·6	5·6
Phloem	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9
Endod-:ermis	—	5·6-4·8	5·6	—	—	—	—	—	—	—	—
Cortex	—	5·9	5·9	5·9	5·9	5·9	—	—	—	—	—
Epid-:ermis	—	—	—	5·6	5·6	5·6	5·6	5·6	5·6	5·6?	5·6?
Palis-:ade	—	—	—	—	—	—	—	—	5·9	—	5·9
Meso-:phyll	—	—	—	—	—	—	5·9	5·9	5·9	5·9	5·9
Epid. hairs	—	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6?	5·6?
Pilif. layer	5·2-4·8	—	—	—	—	—	—	—	—	—	—

	ROOT	BASE HYPO-COT.	TOP HYPO-COT.	BASE EPI-COT.	TOP EPI-COT.	1st INT	2nd INT	3rd INT	4th NODE	4th INT.	6th INT.	PET. 2nd LEAF withered	BLADE 2nd LEAF withered	PET 4th L.	BLADE 4th L.	PET 7th L.	BLADE 7th L.
Pith	—	—	—	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	—	—	—	—	—	—
Xylem	5·2 4·8	5·2 4·4	5·2 4·8	5·6 4·8	5·2 4·8	5·6 4·8	5·2 4·8	5·6	5·6	5·6 4·8	5·6 4·8	5·6	5·6	5·6	5·6	5·6	5·6
Phloem	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9
Endod-:ermis	—	5·6 4·8	5·6	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cortex	—	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	—	—	—	—	—	—
Cortex Fibres	—	4·4	5·2 4·8	5·6 4·8	5·2 4·8	5·2 4·8	5·2 4·8	—	—	—	—	—	—	—	—	—	—
Epider-:mis	—	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6
Epid. hairs	—	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6	5·6
Palis-:ade	—	—	—	—	—	—	—	—	—	—	—	—	5·9	—	5·9	—	5·9
Meso-:phyll	—	—	—	—	—	—	—	—	—	—	—	5·9	5·9	5·9	5·9	5·9	5·9
Pilif. layer	5·6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLE VII. THE XYLEM REACTIONS OF STAGES III - VII.

	III	IV	V	VI	VII
Root	5·2-4·8	5·2-4·8	5·2-4·8	5·2-4·8	5·2 - 4·8
Base of Hypocotyl	5·6-4·8	5·2-4·8	5·2-4·8	5·2-4·8	5·2 - 4·8
Middle of "	5·6-4·8	5·6-(4·8)	5·6-4·8	5·2-4·8	——
Top of "	5·6-4·8	5·6	5·6	5·2-4·8	5·2 - 4·8
Base of Epicotyl	—	5·6	5·6	5·6	5·6 - 4·8
Top of "	—	—	—	—	5·2 - 4·8
1st. Internode	—	—	—	—	5·6 - 4·8
2nd. "	—	—	—	5·6	5·2 - 4·8
3rd. "	—	—	—	—	5·6
4th. "	—	—	—	—	5·6
6th. "	—	—	—	—	5·6 - 4·8
Petiole Cotyledon	5·6-4·8	5·6(5·2)	(5·9) 5·6	5·6	—
Petiole 1st Leaf	—	(5·9) 5·6	5·6	5·6	—
Blade " "	—	(5·9) 5·6	5·6	5·6	—
Petiole 3rd.Leaf	—	—	5·6 (5·2)	—	—
Blade " "	—	—	5·6	—	—
Petiole 4th.Leaf	—	—	—	—	5·6
Blade " "	—	—	—	—	5·6
Petiole 5th. Leaf	—	—	—	5·6	—
Blade " "	—	—	—	5·6	—
Petiole 7th.Leaf	—	—	—	—	5·6
Blade " "	—	—	—	—	5·6

III. DISCUSSION

The Seed

No change in the tissue reactions of the embryo and seed-coat was noted on comparing the results for ungerminated and germinated seeds. This is in agreement with the finding of Martin (1927[1]) for Helianthus Annuus, and Rea and Small (1927) for Vicia faba.

In the embryonic tissues no differentiation of reaction could be established for the different tissue regions. In the first place, the penetration of the indicators was poor, and in the second place it was difficult, on account of the smallness of the embryo, to make hand sections sufficiently thin to enable the regions of this comparatively undifferentiated tissue to be established with certainty. In sections two or three cells thick all parts of the embryo appeared to have the same pH, and were so recorded.

Using germinated seeds with radicles 1 - 1·5 cms. long a number of pH determinations were made to investigate a possible difference in reaction between the tissues of the upper bent part of the radicle and the straight part towards the tip. The indicators however failed to reveal any such difference. Either the tissues are uniform in reaction throughout the length of the radicle, or the variations in pH are of too small an order to be detected by the "Range "Indicator Method".

The/

The Plant

Previous work of a similar nature has been carried out by Martin (1927[1]) on Helianthus annuus, and by Rea and Small (1927) on Vicia faba, while Small (1929) gives some data for Solanum tuberosum. A comparison of the conditions found in Brassica alba with the conditions found in these plants is given below.

Epidermis and Epidermal Hairs. These have a pH of 5·6 at all the stages examined, and in all parts of the shoot. Comparing this with the results of other workers we find somewhat similar reactions in Vicia faba where the leaf epidermis is pH 5·6 - 4·8, the stem epidermis of the green variety pH 5·6, and of the white variety pH 5·2 - 4·8, and in Solanum tuberosum where it is at pH 5·6 at the apex and pH 5·2 - 4·8 towards the base. In Helianthus annuus on the other hand the epidermis is decidedly more acid being in general at pH 4·4 - 4·0, while the epidermal hairs have quite a different reaction varying from pH 4·8 - 5·2 up to pH 9, being in some cases the most basic part of the plant. That the uniformity of reaction found in the epidermis of Brassica alba is not constant for all plants is evidenced by the fact that in Solanum the epidermis becomes more acid towards the base, while in Vicia the reverse is true. In Helianthus although there is no such variation in the reaction of the epidermis of the shoot above ground, the epidermis of the hypocotyl below ground is extremely acid, being

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some cases at pH 3·4.

Cortex - This tissue in Brassica alba appears to have throughout the plant a pH of 5·9. In Helianthus annuus the reaction varies but pH 5·9 - 5·6 is most general for tissues above ground, and pH 5·2 - 4·8 for the hypocotyl just below the soil. In Vicia faba the usual reaction is pH 5·6 towards the apex and pH 5·9 elsewhere. In Solanum tuberosum the reaction varies from pH 5·2 - 4·8 to pH 5·9. The cortex for all these plants shows a tendency to be less acid than the epidermis, and in all cases reaches pH 5·9 at least in some part of the plant. Although there is no difference in reaction between the outer and inner cortex of Brassica alba in Helianthus and Solanum differences were frequently observed.

Fibres of the Cortex - These are only present in the older stages of Brassica alba towards the base of the stem, and in general have a pH 5·2 - 4·8.

Endodermis - The endodermis of the young root of Brassica alba is at pH 5·6. Likewise in the hypocotyl the endodermis tends to be more acid than the tissues immediately surrounding it. This tendency becomes more pronounced the lower one goes in the hypocotyl and the older the plant becomes.

	III.	IV.	V.	VI.	VII.
Middle of Hyp.	5·9-5·6	5·9 (5·6)	5·9	5·6	5·6
Base of Hyp.	5·9-5·6	5·6	5·6	5·6-4·8	5·6-4·8

In Helianthus annuus the endodermis below ground

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is usually at pH 4·4 - 4·0, being thus in general more acid than neighbouring tissues, but in aerial parts of the plant the endodermis does not differ from the adjacent cortical cells and is usually at pH 5·9 - 5·6 or pH5·9 We see in this however the same tendency as in Brassica for the endodermis to be more acid in the lower regions of the plant, though Brassica differs in having its endodermis always more acid than the cortex. In Vicia faba the endodermis is usually at pH 5·2 - 4·8, while the cortex is at pH. 5·6 or 5·9 in the mature plant except at the apex. The endodermis of Solanum tuberosum on the other hand, is recorded at pH 5·9, which is the same reaction as for the inner cortex.

Phloem - This tissue throughout the plant is recorded at pH 5·9. It was yellow to BCP, DER, MR, and BAN and blue-green to BCG and BPB. It was feared that the yellow colour was the natural colour of the tissue and that the four indicators concerned had not penetrated, but the appearance of very deep greenish blue staining with BCG and BPB made it seem more than likely that the other indicators had penetrated and the yellow colour was due to their presence, in which case the reaction of the tissue is 5·9. Martin records the phloem of Helianthus annuus as pH 5·2 - 4·8 with occasional occurrence of pH 5·9. Rea and Small also record pH. 5·2 - 4·8 for the phloem of Vicia faba. In Solanum tuberosum the phloem of the stem above ground and of the leaves is at pH 5·9 as in Brassica alba /

alba, while the phloem of the stem below ground is pH 5·2 - 4·8, and of the root pH 5·6. Thus in neither of these plants is the reaction of the phloem so uniform as it is in Brassica alba.

Xylem - Table VII has been drawn up to show the pH of the xylem in all parts of the plant at successive stages of development. The points to be noted are (1) at any one stage of growth the xylem is more acid towards the base of the plant, (2) for any one region of the plant with the exception of the root the xylem is more acid the older the plant, (3) as the plant grows older the comparatively acid reaction of the xylem extends further up the shoot. The xylem has a uniform reaction throughout the root. Throughout the plant the xylem is as acid as, or more acid than, any other tissue. Martin also finds the xylem of Helianthus to have a relatively high acidity, in this case varying from pH 4·4 - 4·0 to pH 3·4, and refers this to the lignification of the cell walls. The reaction pH 3·4 appears only in the xylem of the stem below soil in the most advanced stages of growth. Otherwise the reaction is uniformly pH 4·4 - 4·0 at all growth stages. In Vicia faba the lignified xylem is at pH 4·4 - 4·0, and the xylem parenchyma generally at pH 5·2 - 4·8. In the mature plant there are a few fibres at the foot of the stem which have a reaction below pH 3·4. This appears to be more or less in agreement with Helianthus. In Solanum also the xylem

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is relatively acid, in this case pH 5·2 - 4·8, throughout the plant. Thus it appears that in Vicia faba and Solanum tuberosum as in Helianthus annuus the acidity of the xylem does not vary much with position in the plant and stage of development. This makes the condition found in Brassica alba all the more noteworthy.

Cambium - Throughout the plant and in all stages the cambium, like the phloem, is at pH 5·9. In Helianthus also the reaction of the cambium seems to agree in general with the reaction of the phloem, in this case usually pH 5·2 - 4·8. The same statement holds for Vicia. The reaction of this tissue in Solanum is given as probably pH 5·6 or pH 5·2 - 4·8 for the middle of the stem, and pH 5·9 for the root.

Pith - This tissue has a constant pH of 5·9 in Brassica alba, a condition not very different from that found in the aerial parts of Helianthus where it is at pH 5·9 - 5·6. In the subterranean parts of this plant however it is at pH 5·2 - 4·8. In Vicia faba also the pith is at pH 5·9 except in the seedling stage when it is pH 5·6 or 5·2 - 4·8, or in the mature stem below ground when it is at pH 5·6. Solanum tuberosum has pith at pH 5·9 - 5·6.

Piliferous Layer. - This varies somewhat in reaction, all values falling between pH 5·6 and pH 4·8. Martin gives the reaction of this tissue in Helianthus as generally pH 4·4 - 4·0. Rea and Small record it for Vicia as generally pH 5·2 - 4·8, occasionally pH 5·6, and sometimes in old lateral roots pH 5·9. The

piliferous/

piliferous layer of Solanum tuberosum is at pH 5·6.

IV. SUMMARY

The tissue reaction of Brassica alba were deter-:mined by means of the "Range Indicator Method".

The plants investigated were grown throughout in a greenhouse at 45-60°F, thus eliminating possible variation due to variation of environmental conditions.

The epidermis and epidermal hairs were throughout at pH 5·6.

The cortex, ground tissue of the petioles, palis-:ade and mesophyll of the leaves were all at pH 5·9.

The fibres of the cortex when present were at pH. 5·2 - 4·8.

The endodermis varied from pH 5·9 to pH 4·8, being most acid at the base of the hypocotyl of the older plants.

The phloem and cambium were at pH 5·9.

The xylem was constantly one of the most acid tissues, being in general at pH 5·2 - 4·8 towards the base of the plant, and pH 5·6 further up.

The pith was at pH 5·9 throughout.

The piliferous layer of the root varied from pH 5·6 to pH 4·8.

The tissue reactions of Brassica alba appear to be subject to much less variation than has been found in other genera by other workers.

A combined list of references for this and the next section is given at the end of the next section.

THE BUFFER SYSTEM OF BRASSICA ALBA

I. INTRODUCTION

The investigation of the buffer systems of plant juices is a comparatively recent development, and so far data is only available for a few species to which reference will be made later. As might be expected, different species vary as to the components of the buffer complex of their sap. Changes have also been detected in the buffering of a single species at different stages of development. Hurd-Karrer (1928) working with wheat plants found that seedlings had titration curves whose forms differed in successive stages of development, and were apparently associated with increasing photosynthetic activity. In a later paper (1930) the change in form of the titration curve of the seedling was attributed to reduction in asparagin content. In the established plant there was increase in buffer capacity with maturation, attributed to increasing concentration of the sap. The same worker (1930) found a difference in the titration curves of etiolated and normal seedlings, which was apparently due to greated asparagin content of the former. Diurnal and seasonal fluctuation in pH of sap have been investigated by various workers, a full account of which is given in Small (1929, Chap. XI).

Deficiency of inorganic salts may or may not bring about alteration of the acidity and buffer com-

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:plex of the sap. Dunne (1932) reports that in wheat plants grown in water culture low phosphate supply causes a slight increase in H.I.C. and an increase of buffering on the alkaline side, probably indicating increase in organic acids, amides, amino acids, and possibly sugars. On the other hand he confirms the findings of earlier workers Dustman (1925) and Newton (1923), that low Ca. supply does not necessarily cause lowering of the pH. Apparently the lack of Ca. is compensated by increased absorption of K.

As regards the buffers of the sap, it seems to be generally agreed that the heat-coagulated proteins do not exhibit any appreciable buffering action. Where proteins do form part of the buffer complex their buffer indexes are small, due to their large molecular weights, Small (1929 p. 75). The most frequently occurring buffers appear to be phosphate and organic acids. In Helianthus annuus phosphate accounts for the entire buffering action of the sap of hypocotyl, stem, and root (Martin 1927[2], 1928[1]). Hempel (1917) finds a high concentration of a single organic acid to be the rule for succulents. Most species however appear to have a much more complex buffer system. Ingold (1929) has identified phosphate, citrate, and malate as the important systems in Solanum tuberosum, with oxalate, asparagin, and tuberin as subsidiaries. In Vicia faba Martin (1928[2]) has identified phosphate, malate/

malate, oxalate, and bicarbonate. Hurd-Karrer (1930) has reproduced the titration curves of wheat seedlings with mixtures containing asparagin, phosphates, sodium malate, glucose, and leucin. She considers the principal buffers to be, between pH 6·0 and 7·5 phosphates, below pH 6 an organic acid complex, and above pH 9·5 leucin and glucose. Armstrong (1929[2]) finds the buffer system of Pelargonium to consist of phosphate, tartrate, oxalate, citrate, malate, and a small amount of unidentified metallic hydroxide. The same writer (1929[1]) has also investigated some of the fungi and reports phosphates, oxalates, and malic acid as the buffers in Coprinus micaceus, and phosphate, citrate, and malate as the buffers of Collybia velutipes.

From this short account it will be seen that the buffer systems of different species vary widely both as to complexity and chemical composition.

II. MATERIAL AND METHODS

A preliminary survey of the constituents of the buffer system of B. alba was carried out, along the lines of Armstrong's (1929[2]) investigation of the buffer complex of Pelargonium. It was intended only as preliminary work for a later investigation when out-door conditions should be suitable for the growth of white mustard in sufficient quantity to enable a more exhaustive and accurate survey to be made.

The plants, from which was derived the sap investigated here, were grown in boxes in a greenhouse at 45-60°F., and were ten weeks old. The shoots were separated from the roots, ground in a mortar, and the sap separated by squeezing through fine strong cotton. The analysis was carried out as described by Armstrong (1929) and Small (1929) appendix I and II. Before analysis the sap, which was dark green when first expressed from the tissues, was filtered through a double layer of filter paper in a Buchner funnel. The filtered sap was pale yellowish-green, and a heavy dark green residue remained on the filter paper.

Two ccs. of filtered sap were used to estimate the inorganic phosphate buffer, and two separate samples of 10 ccs. each to estimate the organic acid constituents of the buffer complex.

The/

The Inorganic Phosphate Buffer

The concentration of inorganic phosphate was determined by Embden's method, (Small 1929, appendix I) By this method the sap is treated with 10% trichloracetic acid, and three volumes of Embden's precipitating agent. This is a freshly made mixture of strychnine nitrate and ammonium molybdate - nitric acid solutions. The precipitate was separated by filtration through a weighed Gooch crucible and dried to constant weight in an oven at 25°C. The amount of inorganic phosphate is determined by dividing the weight of the precipitate by 28·24.

The Tartrate Buffer

One volume of alcohol and half a volume of a 40% solution of potassium acetate were added to two volumes of sap. The precipitate was separated by filtration and treated with hot distilled water which dissolves the tartrate and leaves any protein which may be present. The solution of potassium bitartrate so obtained was titrated with 0·0096 N sodium hydroxide.

The Oxalate Buffer.

Excess 10% calcium hydroxide was added to the filtrate from the potassium bitartrate, and a few drops of fresh lime water added. The resulting precipitate of calcium oxalate was removed by filtration, dissolved in warm 0·1 N sulphuric acid, and titrated with 0·1 N $KMnO_4$ at 60°C.

The/

The Citrate Buffer

A further addition of lime water to the filtrate from the calcium oxalate failed to cause any further precipitation after three days, so that either there was no citrate present, or else it came down with the oxalate. This point needs further investigation. In the presence of excess calcium chloride, oxalates precipitate at pH.5, and citrates at pH 7. The filtrate at this point was amber-coloured so that it was impossible to determine its pH by indicators, and any other method involves the loss of some of the filtrate. The initial pH. of the sap was approximately 5·5, so after the addition of calcium chloride the filtrate was left for some time to see if the oxalates would separate out without the addition of lime water. This did not happen, but on the addition of a small quantity of lime water a precipitate was obtained which was taken to be oxalate. As has been said, no further precipitation occurred on the addition of more lime water, after three days. Presumably no citrates were present.

The Malate Buffer

The addition of 2-3 volumes of methylated spirit to the filtrate caused the appearance of a precipitate of calcium malate in a few minutes. This was separated by filtration through a weighed Gooch crucible, dried at 25°C and weighed.

III EXPERIMENTAL RESULTS

Phosphate Analysis — Table VIII

Sap. Volume	Wt. of ppt.	wt. of H_3PO_4 in ppt.	Molar Concn.
2 ccs	·033 gm.	·00117 gm.	·006

Tatrate Analysis — Table IX.

Sample	Sap Volume	ccs. ·00% N Na OH reqd.	Normality of Bitartrate soln.	Molar Concn.
I.	10ccs.	2·00	·00192	·00384
II.	10ccs	1·95	·00187	·00374
Average	10ccs.	1·98	·0019	·0038

Oxalate Analysis — Table X

Sample	Sap Volume	ccs. 0·1N $KMnO_4$ reqd.	Oxalate Concn. in gm./litre	Molar Concn.
I.	10ccs	0·35	·1575	·00175
II.	10ccs	0·40	·1800	·002
Average	10ccs.	0·375	·1687	·0019

Malate Analysis — Table XI

Sample	Wt. of ppt. in 10ccs sap.	Calcium Malate in gm./litre	Molar Concn. of Malic Acid
I.	·0525gm	5·25	·031
II.	·0555gm.	5·55	·033
Average	0540gm.	5·40	·032

Buffer Complex of Brassica alba Table XII

pH Range	Buffer Indexes for ·006 M Phosphate	·0038 M Tartrate	·0019 M Oxalate	·032 M Malate	Buffer Complex
3·0 - 3·2	·00144	·00291	·00070	·0167	·02175
3·2 - 3·4	·00096	·00291	·00077	·0173	·02194
3·4 - 3·6	·00060	·00304	·00096	·0185	·02310
3·6 - 3·8	—	·00304	·00100	·0185	·02254
3·8 - 4·0	—	·00291	·00108	·0179	·02189
4·0 - 4·2	—	·00291	·00100	·0185	·02241
4·2 - 4·4	—	·00266	·00088	·0191	·02264
4·4 - 4·6	—	·00228	·00077	·0179	·02095
4·6 - 4·8	—	·00152	·00066	·0185	·02068
4·8 - 5·0	—	·00106	—	·0185	·01956
5·0 - 5·2	—	—	—	·0179	·01790
5·2 - 5·4	—	—	—	·0131	·01310
5·4 - 5·6	·00066	—	—	·0107	·01136
5·6 - 5·8	·00099	—	—	·0071	·00809
5·8 - 6·0	·00144	—	—	·0060	·00744
6·0 - 6·2	·00190	—	—	·0047	·00660
6·2 - 6·4	·00238	—	—	·0035	·00588
6·4 - 6·6	·00309	—	—	·0025	·00559
6·6 - 6·8	·00345	—	—	·0019	·00535
6·8 - 7·0	·00345	—	—	·0015	·00495

IV. DISCUSSION

At present no data is available for the erection of a buffer index curve of the sap, to compare with the calculated figures. Consequently all the components of the buffer complex may not have been isolated. The "Range Indicator Method" of pH estimation could not be employed, owing to the fact that the filtered sap had a yellowish-green colour, which obscured the reactions of the indicators. The only electrical apparatus available was of a new type which is still at the experimental stage.

From table XIII it appears that, of the substances isolated, over the range pH3 to pH7, malate is responsible for most of the buffering effect at the more acid end, and phosphate for most of the buffering effect at the less acid end of the range. This agrees to some extent with Armstrong's figures for Pelargonium, in which malate accounts for 46·6% of the observed buffering at pH 3·0 - 3·2, and phosphate for 37·8% at pH 6·8 - 7·0. On the other hand the oxalate content is very much smaller in Brassica alba than in Pelargonium.

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THE EFFECT OF THE MUCILAGE OF THE SEED-COAT IN GERMINATION OF BRASSICA ALBA

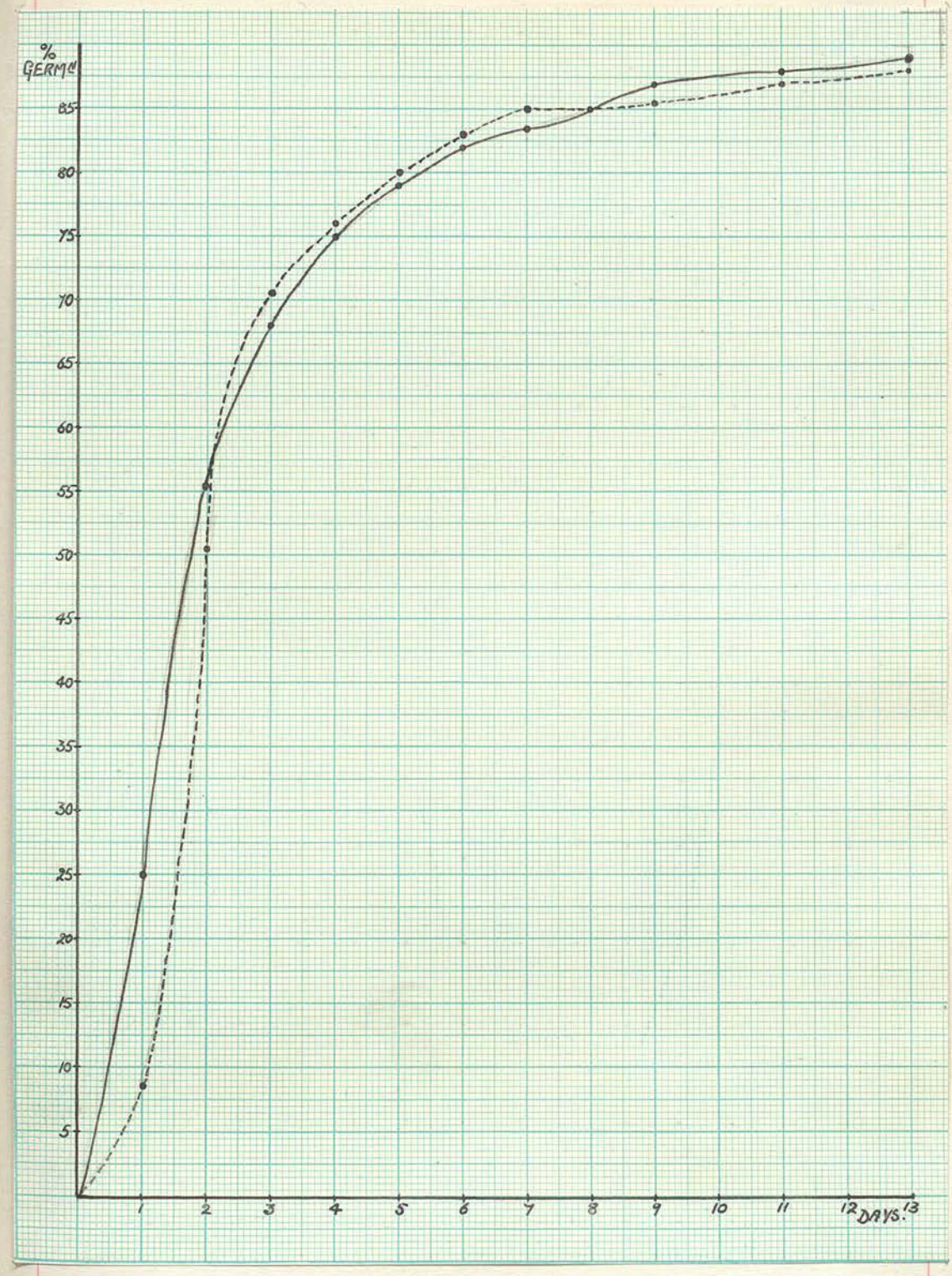
This investigation was carried out in order to examine the effect of the mucilage in the seed-coats of B. alba with regard to germination. When the seeds are soaked in water within a very short time they become slimy due to the rapid absorption of water by the mucilage held in the outer layers of the testa. It was thought that if this mucilage could be removed and the treated seeds germinated, some light might be thrown on the possible role of the mucilage in germination.

The precipitation of the mucilage by electrolytes was the first line of attack, and it was found that a 5% solution of Barium chloride did indeed bring about precipitation, but that the precipitated mucilage adhered to the seed-coat. This may have been due to the fact that the mucilage was only partially precipitated, and sufficient was left in a colloidal condition to cause the flocculated portion to adhere to the seed-coat. Removal of the mucilage was eventually effected with methylated spirits. When the spirit was poured over the soaked seed in a shallow porcelain dish the mucilage swelled suddenly within a few seconds and formed a relatively enormous mass of transparent gel. When the/

the seeds were removed to a test tube and shaken up with the spirit this gel precipitated and separated from the seeds.

A series of germination tests were made using seeds soaked in water for 3, 6, 9, 12, 15, 18, 21, and 24 hours before treatment with methylated spirits. The seeds were germinated on pads in an electrically heated germinating tank, in the same way as has been described for Aira flexuosa. The tests were run in light at 20°C constant temperature. Each batch of treated seeds was accompanied by a control batch, soaked in water for the same length of time but not treated with methylated spirits. Each treated batch and each control consisted of two pads, each with 70 seeds. The germinating seeds were counted at regular intervals of 24 hours for the first week. After that time germination had become very slow and counts were sometimes made after 48 or 72 hours. The results are given in table XIII, each figure being a percentage calculated from the average of each pair of comparable pads. The figure given for any particular day represents the total percentage germination up to that day.

Length of soaking in water		% Germination in														% Part. Plum. Dev.	% Total Plum. Dev.
		1 day	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.		
3 hours	Treated	0.	51.	70·5	82.	90·5	93.	94.	95.	95.	-	-	95.	-	95.	13·5	15.
	Control	5·5	63.	73·5	83.	85·5	88.	88·5	90·5	90·5	-	-	93·5	-	93·5	12.	11·5
6 "	Treated	0.	20·5	49.	60.	70.	76·5	80.	83.	87.	-	-	88.	-	88·5	17.	9.
	Control	8·5	53.	68·5	76·5	80.	83.	85·5	85·5	86·5	-	-	88.	-	88.	14.	16·5
9 "	Treated	0.	40·5	66.	75.	83·5	86·5	88.	-	-	92.	-	93.	-	-	13.	9.
	Control	10.	55.	70·5	78·5	83·5	88·5	90·5	-	-	93.	-	94.	-	-	15.	15·5
12 "	Treated	0.	20·5	45·5	58.	62.	65·5	70·5	-	77.	-	79.	-	-	-	18.	17.
	Control	8·5	42.	54.	60·5	64.	67.	69.	-	75·5	-	79.	-	-	-	8.	18·5
15 "	Treated	2.	42.	60·5	68.	78·5	82.	84.	87.	89.	-	90·5	-	92.	-	3.	13.
	Control	24.	62.	70·5	76.	80.	82.	83.	84.	86·5	-	86·5	-	88.	-	2.	9.
18 "	Treated	8·5	50·5	70·5	77.	80.	83.	85.	85.	85·5	-	87.	-	88.	-	20.	12.
	Control	25.	55·5	68.	75.	79.	82.	83·5	85.	87.	-	88.	-	89.	-	21·5	17.
21 "	Treated	2.	40.	60·5	72.	78·5	85.	88.	90.	-	90·5	-	91·5	-	-	23·5	18.
	Control	2.	46.	63.	68.	74·5	78.	79.	80·5	-	83·5	-	85·5	-	-	19.	13·5
24 "	Treated	0.	8.	57.	71·5	77.	81·5	84.	-	-	90.	-	92.	-	-	18·5	14.
	Control	0·5	41.	64.	70·5	73·5	78·5	80·5	-	-	86·5	-	88.	-	-	18.	15·5



Germination of Brassica alba

Fig. 1. Seeds soaked for 18 hours

—— control

---- treated

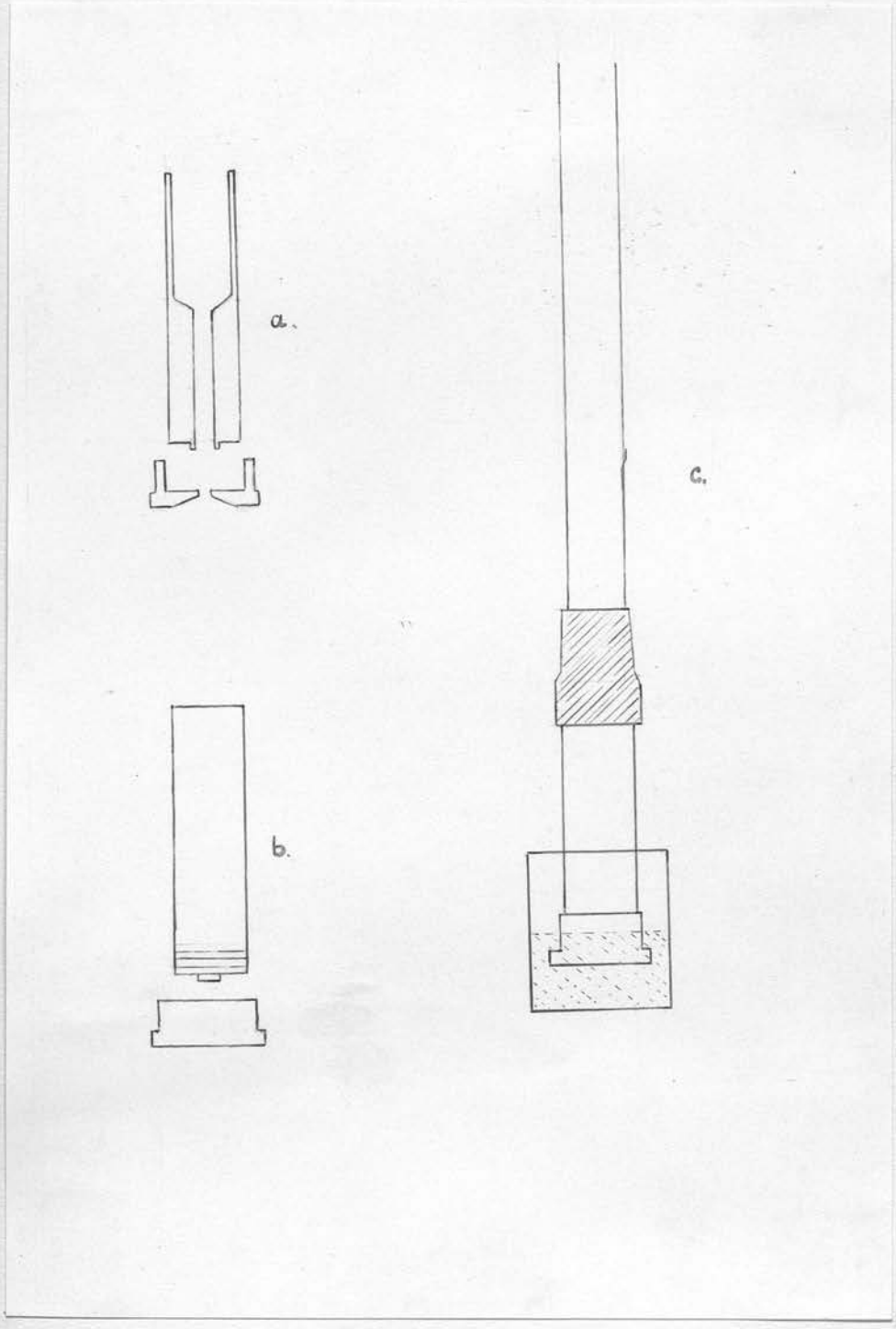


Fig. II. Apparatus for measurement of semi-permeability of seed-coat.

Table XIII. shows that the germination of seeds, from which the mucilage has been removed by treatment with methylated spirits, is appreciably slower than the germination of untreated seeds in the first few days of germination, and that it subsequently becomes more rapid so that a few days later the total percentage germination of treated seeds is generally greater than the percentage germination of untreated seeds. The exact time taken by the treated seeds to show germination in excess of untreated seeds varies considerably and does not seem to bear any relation to the length of previous soaking in water. Thus in the series from 3 hours to 24 hours soaking, the germination of treated seeds caught up with that of untreated seeds in respectively 5, 9, 5, 7, 7, 3, 4, and 4 days. In one case only did the percentage germination of treated seeds at no stage exceed the percentage germination of untreated seeds. This was in the 3rd. batch (9 hours soaking). But even here the germination of the treated and untreated seeds was equal by the 5th day. Towards the middle of the second week in most cases there was a slight tendency for the untreated seeds to catch up with the treated ones. In a single case (18 hours soaking) the percentage germination of the untreated seeds was eventually higher than the percentage germin-:ation of the treated seeds. This is illustrated in Fig. 1. by a graph which shows the curves of germination of treated and untreated seeds crossing each other on

the/

the 3rd day and again on the 9th day.

The reactions involved in these results are obscure, and it is difficult to place proper emphasis on each factor. In the first place it might be said that the excess of germination percentage of treated seeds in the second stage of germination is not sufficiently great to be of much significance, 9% being the greatest difference observed. But this is surely overruled by the fact of its constant occurrence throughout the whole series of tests.

The semi-permeability of the treated and untreated seed-coat was investigated to determine what effect if any was produced by the precipitation of the mucilage. For this a special apparatus was used, consisting of a brass cylinder 1 cm. in diameter, open at both ends, the walls of the upper half being about 1 mm. thick, and of the lower half about 3·5 mm. thick, so that the aperture at this end is 3 mm. in diameter. This aperture has a projecting rim over which the seed-coat is stretched and held in place by a screwed on cap with an aperture of 2 mm. in the centre. A glass tube fitted into the upper half of the cylinder and held by a short length of rubber tubing completes the apparatus. (Fig. II. a, b, c.)

The cylinder was filled with a concentrated solution of sucrose and held in place with the end dipping into distilled water, so that the seed-coat formed a membrane between the two liquids. The untreated seed/

seed-coat showed a slight degree of semi-permeability, there being a rise of 2 mm in the glass tube of about 5 mm. bore, in 3 days. The distilled water was tested at the end of the experiment for reducing and non-reducing sugars, with negative result. The treated seed-coat on the other hand appeared to be quite im-:permeable, since there was no rise in the level of the sucrose solution after 7 days, and no trace of sugars could be detected in the distilled water.

A microscopic examination of the treated and untreated seed-coat failed to bring out any marked difference between them.

A certain percentage of the germinated seeds showed that type of abnormal growth called plumular development. In some cases the radicle remained totally undeveloped, in others there was a slight development giving rise to a short radicle with a few root hairs. The occurrence of this abnormality is recorded in the last two columns of TableXIII. It appears to be unaffected both by removal of the mucilage of the seed coat, and by length of soaking in water previous to germination.

The effects of other alcohols, and mixtures of alcohols, on the soaked seed, were tested. Some of them caused slight swelling of the gel and precipitation, but in no case was the effect so pronounced as that produced by methylated spirit. In descending order of activity the following had some effect (1) a

mixture/

mixture of methyl alcohol, ethyl alcohol, and water, in approximately the same proportions as they occur in laboratory methylated spirit (2) methyl alcohol (3) ethyl alcohol. Amyl alcohol had no effect whatever.

It is perhaps of interest to note that Russell (1919) working with camphor seeds found that removal of the pulp gave, under commercial conditions, both quicker and better germination, larger and sturdier seedlings, besides a large increase in the number of seedlings to attain transplanting size.

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THE DISTRIBUTION OF STARCH IN THE RADICLE OF BRASSICA ALBA

Germinated seeds with radicles 1-2 cms. long were tested for oil, protein, and starch. A heavy protein reaction with Millon's reagent was given throughout the length of the radicle. In the case of starch, however, the distribution was not uniform, and had some features of particular interest.

When radicles showing curvature in the part furthest from the tip, Fig. III a, were sectioned longitudinally and stained with iodine a concentration of starch was found in the endodermal region of the bent part, but not of the straight part, of the radicle. Radicles which had grown in a continuous curve, Fig. III b, showed this starch sheath throughout their length, except at the undifferentiated tip. To confirm these results, and obtain a more accurate record of the starch distribution a number of radicles were embedded in wax, and sectioned with the microtome. In all cases the results were confirmed. A small quantity of starch was present in the cortical cells throughout the length of all radicles. A heavy starch content however only occurred in the one or two rows of cells immediately surrounding the central stele in any part of the radicle which was definitely curved. Such concentration of starch as was present at the root tip was confined mainly to the periphery. The facts

seemed/

seemed to indicate that the presence of starch in the endodermal region had some connection with geotropic response.

Attempts to discover an endodermis in the young radicle, by dissolving the tissue in concentrated sulphuric acid (Priestley and North 1922) yielded negative results. Priestley (1926) says "In the shoot (Vicia and Pisum) a starch sheath appears at an early stage in development. In the light it remains, in the dark it is transformed into a primary endodermis. In the root a primary endodermis arises out of the meristem cells as they differentiate, a starch sheath is never formed". If this is the case in Vicia and Pisum there is no reason to suppose that the starch sheath in the radicle of B.alba has any connection with the formation of an endodermis, especially in view of the fact that it arises only where the radicle shows geotropic curvature.

In an earlier paper Priestley and Ewing (1923) give a fuller account of the occurrence of a starch sheath. "In the normal Angiosperm stem when growing "in the light a functional endodermis with casparian "strip is usually not present, its place being taken "by the "starch sheath". - - - The stem of the etiolated "broad bean seedling possesses a fully developed, "functional endodermis from its base to close behind "the growing apex". The authors expound the theory that in the stem grown in the dark the endodermis developed/

developed under these circumstances largely restricts the supply of nutrient material to tissues within the endodermis and to that part of the apical meristem which caps the endodermal cylinder, so that growth in length takes place at the expense of growth in breadth.

Philip-Smith (1928) makes reference to the occurrence of a starch sheath in the stem of Clematis. "The starch sheath begins to differentiate in the "apical meristem about the level of the first bud "primordium - - - . Once the starch sheath appears "the distribution of starch in the young stem is "influenced by it. In the young and rapidly growing "stem little starch is deposited in the tissues, and "what does occur is confined to the cortex - - - . "After rapid growth has ceased in the internode starch "gradually accumulates in all the tissues, intrastelar "as well as extrastelar, and it is no longer possible to "distinguish the starch sheath as a morphological "boundary - - -. The question arises, how far can "this starch sheath be considered as equivalent to "an endodermis? Considered as a barrier to the passage "of starch (or rather of those soluble carbohydrates "from which starch may be formed), it is evidently "functional only when the excess of carbohydrates in "the stem is not great. The fact that, once active "growth in length has ceased, starch accumulates in "great quantity in the pith shows that the starch "sheath is only effective within narrow limits. There "is/

"is no sign of a Casparian strip in any normal stem. "Attempts to induce its development by etiolation, "both in strongly growing young shoots and in seedlings, "were unsuccessful. It may therefore be said that "neither structurally nor functionally is the starch "sheath of Clematis fully equivalent to an endodermis."

In an earlier paper (Philip Smith 1923-4) the same writer, while investigating the propagation of Clematis, found that rooting at the node only took place after etiolation. "A comparison of the etiolated "with the normal stem showed a great decrease in the "amount of starch present. The xylem parenchyma, the "pith, and the medullary rays were practically starch "free; the starch-sheath was even more prominent than "usual, and the amount of starch in the cortex was greatly "reduced. In order to examine the possible relation of "the starch sheath to an endodermis, the tip of a strongly "growing shoot of C. Forestii was enclosed in a black "paper bag and allowed to grow in the dark for four "weeks. No evidence of a Casparian strip could be "obtained in this or any of the species examined, so "that the starch sheath cannot be considered as truly "equivalent to an endodermis. In any case, the "behaviour of the blanched cuttings shows that it has "little or nothing to do with the relative ease of "rooting."

It is clear that in the first two cases quoted, the presence of a starch sheath coincides with active radial/

radial growth of the cortex. In the etiolated bean the restricted radial growth of the cortex is accompan-:ied by the appearance of an endodermis in place of the starch sheath of the normal stem. When the Clematis stem ceases active growth the starch sheath disappears. In the radicle of B alba the starch sheath coincides with curvature. Here again it is associated with active cortical development, since the cortex is the tissue which must develop most rapidly if curvature is to take place. It is true that the starch sheath forms a complete cylinder while cortical development is particularly active only on one side of the radicle, but this does not necessarily invalidate the thesis developed here. If we have not sufficient understanding of the causes and direction of movement of food in plant tissues to enable us to explain why and how the starch sheath is formed, and until we understand more about geotropic stimulus and the response of the plant to it, it is impossible to say whether or not this is a serious objection to the ideas advanced here. At present we can only try to correlate the presence of a starch sheath with some activity or activities of the plant, from observed facts.

In the last case quoted (Philip-Smith 1923-4) etiolation of the node of Clematis causes the starch sheath to become more prominent than in the normal stem. This is accompanied by greater ease of rooting, but in the author's opinion the two facts are uncon-:nected. It is suggested here that the starch sheath may/

may have some bearing on rooting, not indeed directly, but indirectly through its influence on the formation of callus. Earlier in the same paper Philip-Smith observes that in all cases the first indication of callus formation was the beginning of meristematic activity in the cortex. It is significant that once more a starch sheath is found in conjunction with activity in the cortex.

Hitherto all the emphasis has been laid on the presence or absence of the endodermis, the starch sheath being considered significant apparently only when it takes the place of an endodermis, or, if no connection with an endodermis has been demonstrable, dismissed as of no importance. However the very fact that a starch sheath has been recorded both when it replaces an endodermis and when no connection with an endodermis can be establised, lends weight to the opinion that the starch sheath plays some positive role in the particular circumstances in which it appears. The obvious explanation of the presence of starch at a region of active growth is as a readily available food supply. But the explanation can not be so simple. In the first place it is obvious that it is not so much the mere presence of starch, as its concentration in a particular region, which is import-:ant. For instance, after the stem of Clematis has ceased active growth the starch sheath disappears, but the actual amount of starch in the stem tissues is

greatly/

greatly increased. We are therefore forced to the conclusion that it is the region of concentration of the starch which is significant. The question still remains, is it a food supply, or a regulator of food supply? Philip-Smith (1928) seems to incline to the latter view, apparently because it occupies the same position as the endodermis, but even she is doubtful. It is difficult to see how it could possibly fulfil this function. Although the starch content of the cells is high, the cells are by no means choked with it, and it is unlikely to interfere to any great extent with diffusion of substances through the protoplast. As no casparian strip is present the walls must allow free passage to water and dissolved substances,

The most likely view is that the starch sheath is a convenient temporary store for food material essential for the active growth of the tissues of the region in which it occurs. It is convenient for two reasons, firstly that it is adjacent to the vascular tissue which translocates the food and secondly because its position is such that it can supply food most readily to the peripheral tissues of the stem which are those that owing to their position are unable to tap the supply from the vascular tissue direct.

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EXPLANATION OF PLATES

Fig. III. a,b. Distribution of starch in young radicle of B. alba.

Figs. IV - VIII. Transverse sections of a radicle of B. alba, of type shown in Fig. IIIa, showing starch distribution at successive levels beginning near the tip. Note the great increase of starch in endodermal region of the bent upper portion.

Fig. IX. Photograph of L.S. of radicle of B. alba, of type shown in Fig. III a, showing concentration of starch in the endodermal region of the curved part.

Fig. X. Photograph of T.S. of radicle of B. alba (as in Fig. III a) Section made through bent part to show starch sheath.

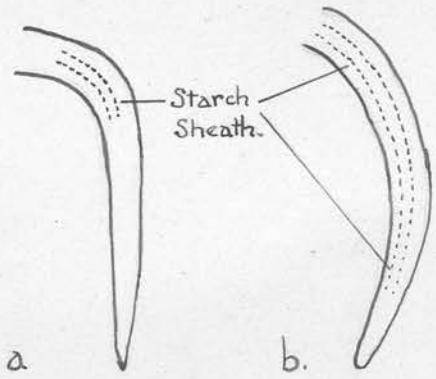

FIG. III

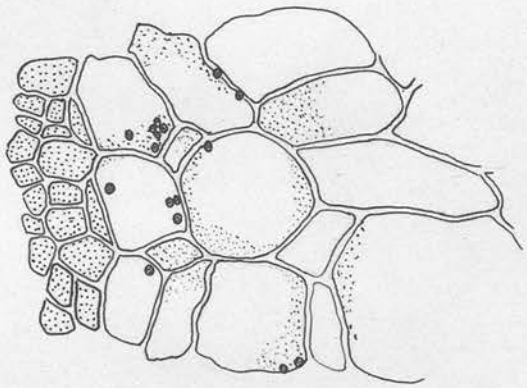

FIG. IV

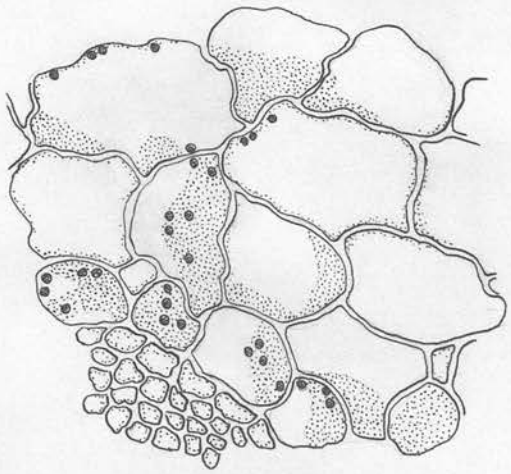

FIG. V

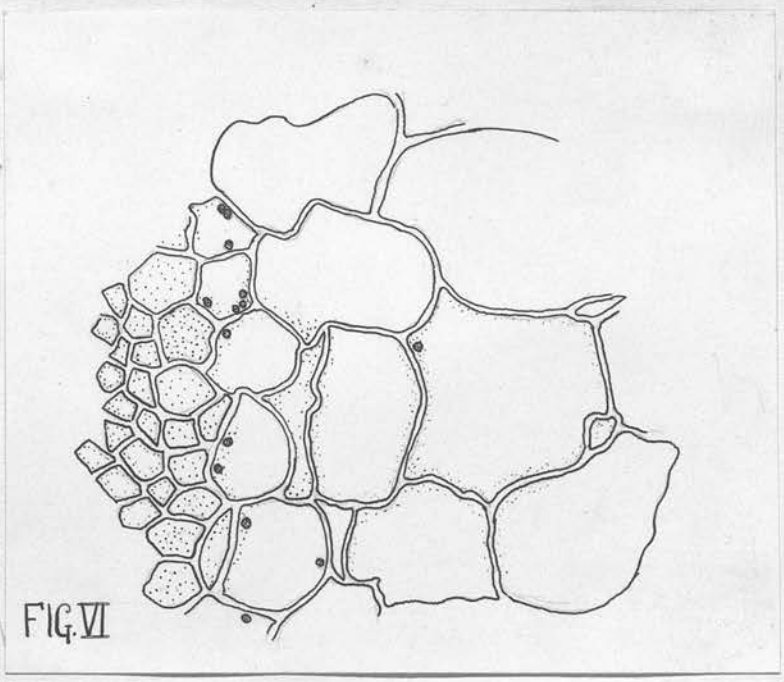

FIG. VI

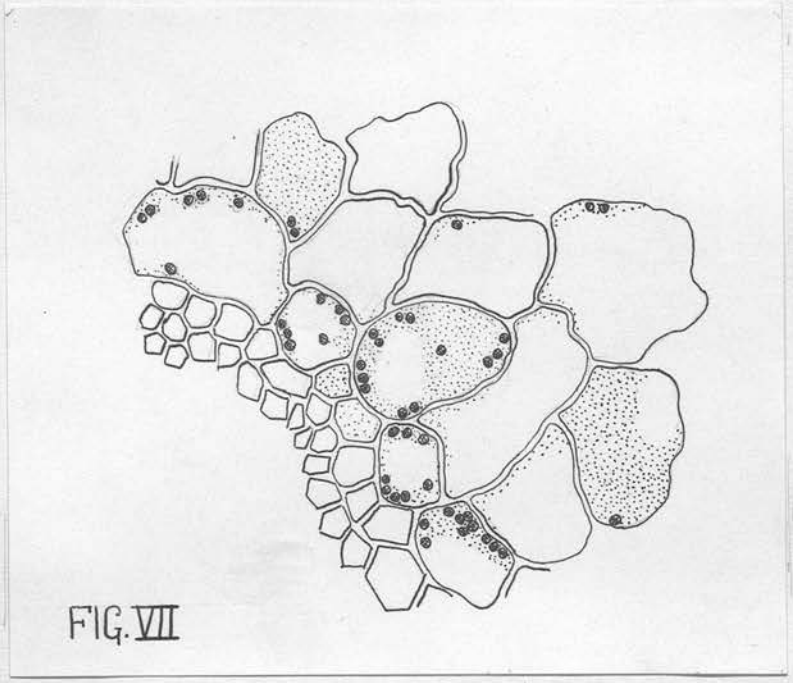

FIG. VII

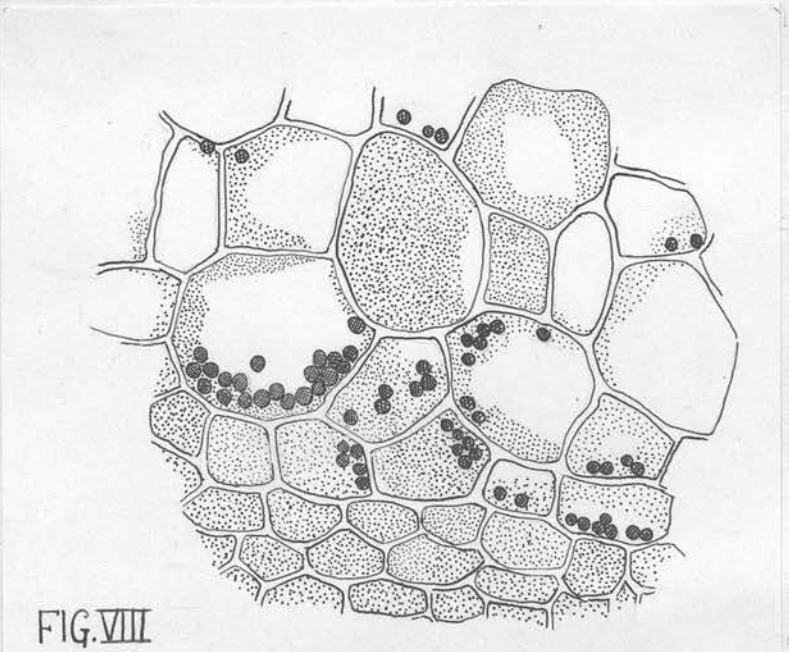

FIG. VIII

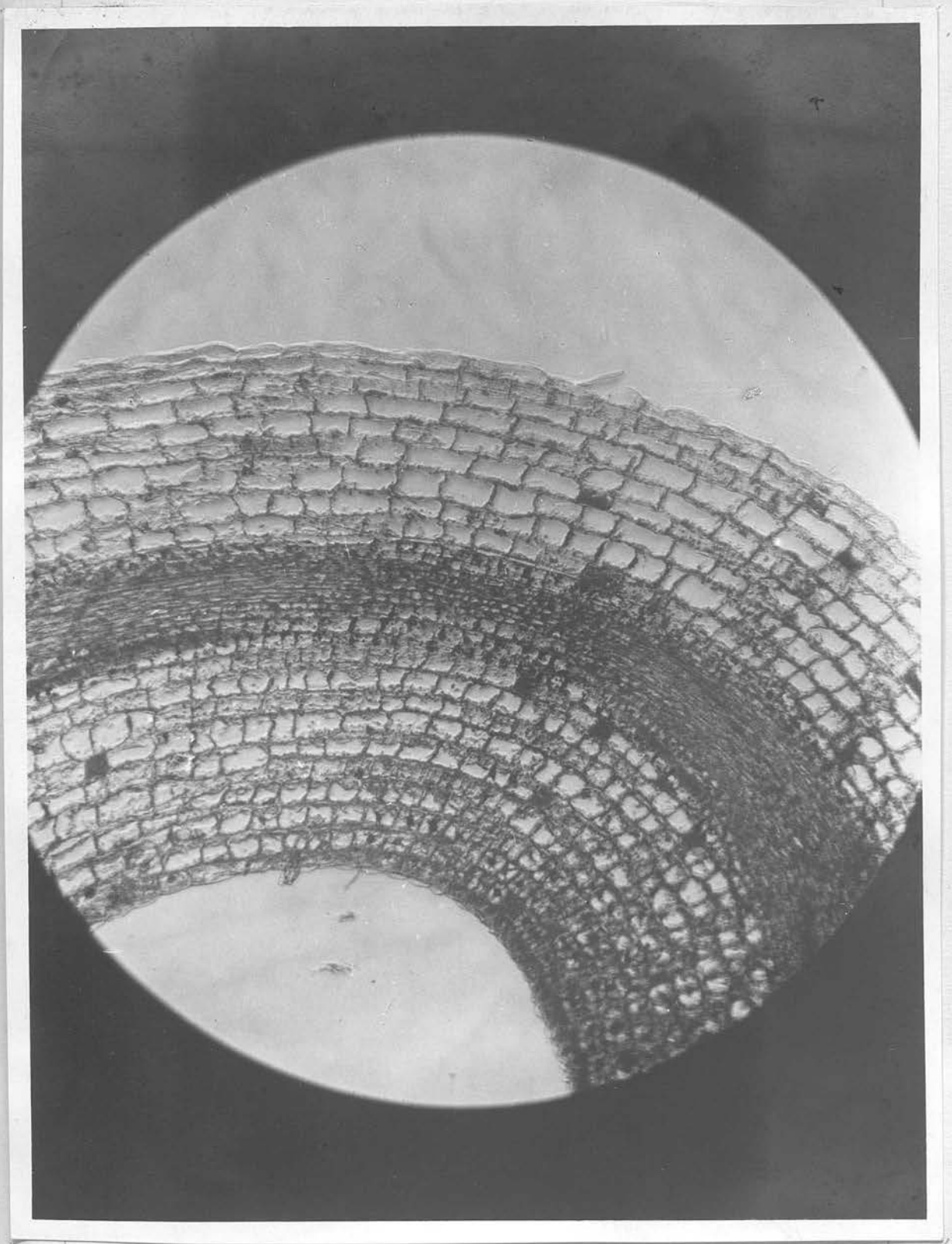

Fig. IX.

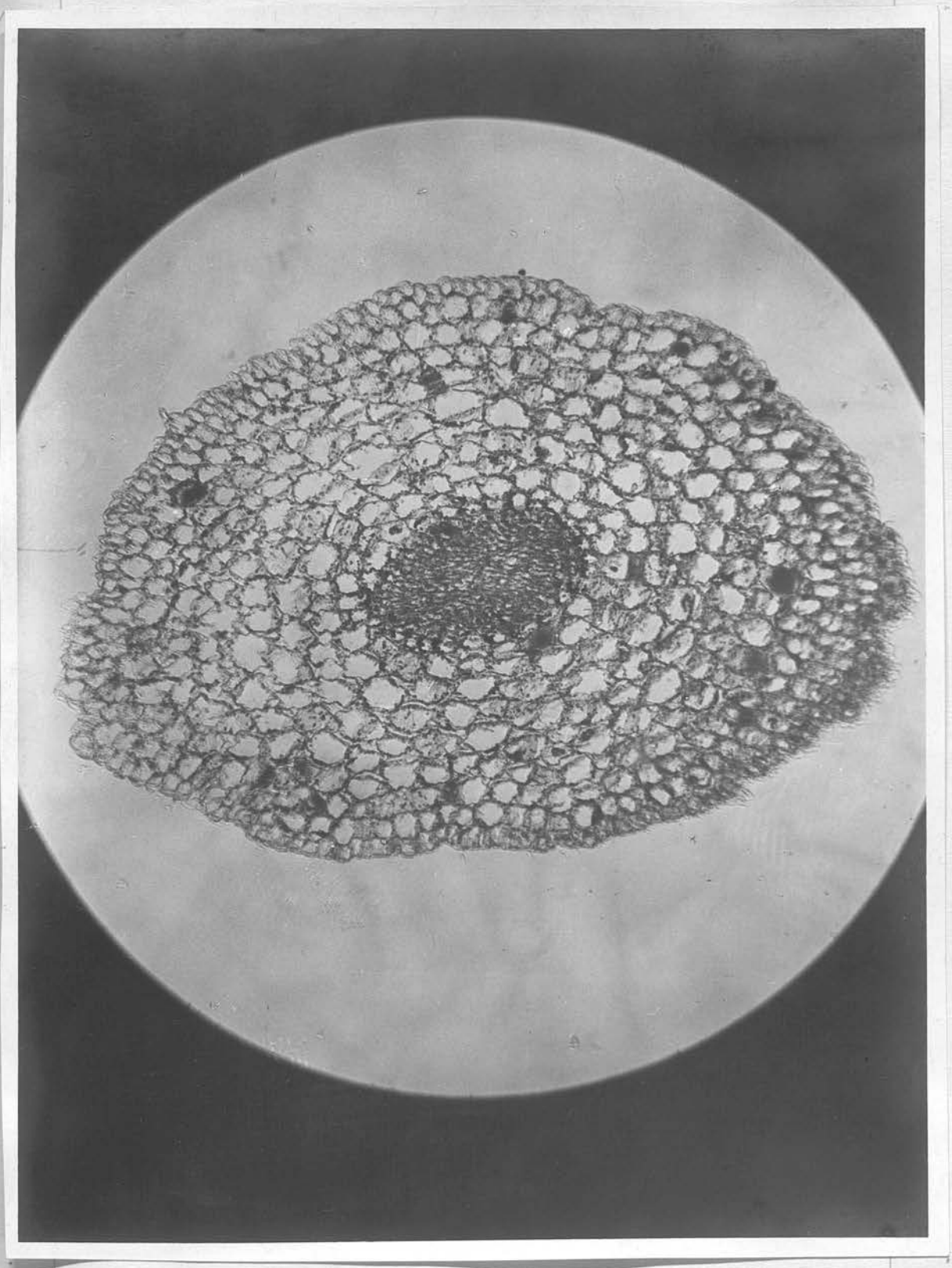

Fig. X.